AGRICULTURAL PUBLICATIONS
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GENETICS
LABORATORY MANUAL

McGraw-Hill Book Co. Inc.

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GENETICS LABORATORY MANUAL

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FIRST EDITION

SECOND IMPRESSION

McGRAW-HILL BOOK COMPANY, INC.
239 WEST 39TH STREET. NEW YORK

LONDON: HILL PUBLISHING CO., LTD.
6 & 8 BOUVERIE ST., E.C.

1918

THE MAPLE PRESS YORK PA

PREFACE

Carefully planned laboratory instruction is as important in the teaching of genetics as in any other phase of natural science. The value to the student of first-hand acquaintance with the phenomena of variation and heredity must be obvious. But the selection of the most suitable material for a single half-year course in genetics involves some problems that are not met in other branches of biology. For example, the cost of providing large classes with the necessary material for the study of variation and Mendelism must be considered. Such animals as mice, rats, guinea-pigs, rabbits and the domestic fowl furnish excellent material, but a sufficient quantity is available only at institutions having the necessary facilities for rearing large numbers and preserving specimens properly. The number of such institutions is comparatively limited. On the other hand, the cost of producing a sufficient quantity of cultivated plants for either variation or Mendelian studies is little more than nominal, and many wild plants furnish excellent material for the study of variation.

The time element is also an important consideration. In this connection it is fortunate indeed that the one animal which has yielded by far the most important data on the mechanism of heredity, the vinegar or fruit fly, *Drosophila melanogaster* (*ampelophila*), embodies nearly every advantage that could be desired by the genetics instructor. Fortunately some plants also can be utilized for actual breeding experiments involving the F_2 generation by making the original cross and growing the F_1 generation ahead of time, and providing the class with F_2 seeds at the beginning of the course. For the most satisfactory culture and preservation of necessary plant materials some greenhouse facilities are almost indispensable, yet the total lack of such facilities need not deter instructors from including actual plant-breeding exercises. Cereals, garden peas and sweet peas can be grown out-doors in nearly every climate and by proper preparation of material during the year or two previous the study of hybridization and hybrid material in those plants can be made a very valuable part of the course. Similarly with Boston ferns and other plants that are best grown in the greenhouse, if plants or fronds cannot be secured from some local nurseryman, pressed specimens can be prepared at a florist establishment making a specialty of these decorative plants and such material can be used over and over again by having it properly mounted.

The advantages of alternating the materials used in a laboratory course so that the same materials are not used two years in succession must be patent to every genetics instructor of experience. Especially is this the case with exercises involving Mendelian ratios and mathematical calculations of any kind. For this reason, as well as for the purpose of meeting as many conditions as possible, we have suggested three alternative exercises under most of the numbers. The work outlined, therefore, is sufficient for three half-year courses consisting of one 3-hour period per week for 15 or 16 weeks. By slight modification and amplification the exercises can be adapted to a course calling for two or three periods each week. Nearly every instructor will, of course, have some material of his own which he will prefer to use and the general scheme of arrangement is sufficiently elastic to allow for its introduction. We believe that the courses herein provided conform to sound pedagogical principles in arrangement and method and that the most fundamentally important genetical principles and laws will be mastered by the students who satisfactorily complete one of these courses.

It is assumed that the laboratory course will accompany and supplement a combined lecture and recitation course and this manual is intended in particular to supplement the text-book, "Genetics in Relation to Agriculture," by Babcock and Clausen. Although it may seem advisable in some cases to alter the sequence of laboratory exercises according to the progress of the class in the study of subject matter in the text and lectures, yet it may be found more feasible to assign certain portions of the text to precede, accompany or closely follow certain laboratory exercises. Probably the latter would be the wiser plan inasmuch as the laboratory exercises may occasionally have to be shifted in order to take advantage of a certain stage of development in plants or for various other reasons.

With these provisions in mind the following series of exercises are recommended. The exercises referred to here by number will be found in proper sequence in the body of the manual. It will be remembered that under each number there are three alternatives designated as *a*, *b*, and *c*. If the "*a*" series of exercises is used in 1919, the "*b*" series may be used in 1920 and the "*c*" series in 1921; or by selecting and rearranging individual exercises an instructor can make up an entirely different course of one, two or three laboratory periods per week as desired. Certain exercises, however, call for material which can hardly be duplicated and it is recommended that they be included in each year's work. Further suggestions and various specific aids to laboratory work will be found in the appendices.

Following is the series of exercises which has been found most satisfactory in the experience of the authors:

First week, 1; second, 2 and 7; third, 3 and 15; fourth, 4 and 16; fifth, 5 and 11; sixth, 6 and 12; seventh, 13; eighth, 14; ninth, 9; tenth, 10; eleventh, 8; twelfth, 17; thirteenth, 18; fourteenth, 19; fifteenth, make up or repeat necessary work.

It will probably be necessary to modify the sequence given in the above series according to the development of living plant materials.

Acknowledgments.—To our coworker, Dr. R. E. Clausen, we are indebted for many valuable suggestions in planning the laboratory exercises. Especial acknowledgment is also due the following: to Dr. Raymond Pearl for the probability table (Table IX); to Dr. G. H. Hart for the certificate of registry shown in Fig. 6; to Miss C. J. Hill for copies of official Holstein-Friesian certificates (Figs. 9–12); to the Holstein-Friesian Association of America and the American Shorthorn Breeders Association for application and registration blanks; to Professor A. W. Gilbert for data on *Pisum sativum;* and to the *Journal of Heredity* for Fig. 3.

THE AUTHORS.

BERKELEY, CALIFORNIA,
May 17, 1918.

GENERAL DIRECTIONS FOR STUDENTS

Students will provide themselves with the following unless otherwise instructed:

Babcock and Clausen: "Genetics in Relation to Agriculture."
Notebook for lectures.
Notebook for laboratory, size 8½ in. × 11 in., containing blank paper for notes and drawings and coördinate paper.
10 manila folders for laboratory reports.
1 box Dennison gummed labels No. 217.
24 brass paper fasteners, No. 4, 1 inch long.
1 hand lens.
1 dissecting forceps.
2 dissecting needles.
1 scalpel.
1 small metric rule or steel tape.
The other apparatus and materials needed for this course will be furnished.
Students will be held responsible for the preservation of this material in good condition.

General Plan of Laboratory Course

The work of this course will consist of four lines of study as follows:

1. Breeding experiments with the vinegar fly.
2. Study of variation in plants.
3. Work with material illustrating the Mendelian principles.
4. Study of some features of plant and animal breeding.

The laboratory exercises are arranged in the four above-named groups, but it is impracticable to study only one group at a time and cover the whole course in the allotted time. Therefore the work of a single laboratory period may include an exercise from two or more of the above groups. A progressive series of combined laboratory exercises is suggested on p. vii. It is easily modified, however, to suit particular needs and conditions.

CONTENTS

GENETICS LABORATORY MANUAL

LABORATORY EXERCISES

I. DROSOPHILA BREEDING EXPERIMENTS

General directions for conducting experiments with *Drosophila melanogaster* (*ampelophila*).

A. Object.—The object of these experiments is to lead each student to a correct understanding of the operation of certain laws of heredity in the common vinegar fly. Independent observation and reasoning on the part of students will be constantly encouraged throughout the progress of the experiments. The various mutations of this fly have proved to be very useful for demonstrating the nature of Mendelian inheritance and they are used in this course in order to give a first-hand knowledge of such phenomena.

B. Methods.—The methods of handling the flies and conducting experiments outlined in these general directions and in the laboratory exercises are those which have been found best adapted for use in large classes. They are not ideal, but, if properly followed, they will give satisfactory results. For the methods used in research work see appendix II.

C. Pure Strains.—The eight pure strains of flies called for in these experiments involve the following characters:

1. Body colors—gray, black, yellow, ebony.
2. Wing characters—long, miniature, vestigial.
3. Eye colors—red, white, sepia.

The names used are the same as those used by Morgan and his associates in the various books and articles in which they have described their experiments, and the characters are known to behave strictly according to the manner therein represented. For the sake of brevity the characters are designated by initials as follows:

B	black	*R*	red
E	ebony	*S*	sepia
G	gray	*V*	vestigial
L	long	*W*	white
M	miniature	*Y*	yellow

The pure strains are conveniently designated by the initials corresponding to the mutated characters represented in them. Thus Strain *B* is a black-bodied race; Strain *BMW* is a black-bodied, miniature-winged, white-eyed race, and so on. For the sake of uniformity the following order of characters is preserved throughout; body color, wing character, eye color. Of the above list of characters, *gray body, long wings,* and *red eyes* are *not mutation characters,* but are the characters of the wild, normal fly. The normal fly with no obvious mutated characters is called a wild type or plus fly, and is often simply designated by the + symbol. This system of designation should not be confused with the symbolic representation of the genetic constitutions of the flies or their gametes; it is merely a convenient way of describing the outward appearance of the flies, whether of pure strains or isolated individuals.

Pure strains of these flies breed true as long as maintained separately. However, mutations may be found occasionally in any pure culture. The occasional occurrence of mutations should stimulate the student to search for them.

D. Materials.—For class purposes, homeopathic vials of 8-drachm (30-c.c.) capacity are used for breeding bottles. They are fitted up with towel paper and cotton plugs and dry sterilized before being used. Flies are ordinarily classified as soon as they are removed from the breeding vials; but, if it is found necessary they may be preserved in strong alcohol in 1-drachm (4-c.c.) homeopathic vials. Each student is furnished with a wooden tray for the necessary vials (see Fig. 13) and this tray should bear a label with the student's name, class number and laboratory section. Since these experiments extend throughout the semester, it is necessary that the breeding outfit be kept clean and neat.

E. Food and Moisture.—Yeast-fermented banana is the ideal food for laboratory purposes. This is furnished to the students in breeding vials in order to prevent contaminations which are likely to occur in a common supply. This food supplies sufficient moisture while in a fermenting condition, but as it becomes old and dry there will not be enough moisture to sustain life. The dry condition is best remedied by adding a new supply of fermenting banana to the vial. Observe great care about this or the culture will dry up, and the yield will be correspondingly low. Cultures which have been kept in good condition will give at least 50 flies in each vial, and any number less than this should be regarded as unsatisfactory. Cultures should be examined two or three times a week. It will not be necessary to feed them as often as this, but the student should be sure they are in good condition.

Certain kinds of bacteria sometimes infect the cultures and produce a slimy growth over the surface of the banana. The flies will not breed satisfactorily in such cultures, and if transferred to a fresh supply of banana they will carry the bacteria with them. Accordingly show sus-

pected cultures to the instructor. Ordinarily contaminated cultures should be discarded immediately and no flies or pupæ should be taken from these to establish other cultures.

F. Handling the Flies.—The flies are positively phototropic; that is, they tend to move toward a source of light. When, therefore, it is necessary to supply fresh food or moisture, the vial should be held horizontally with the open end away from the window. When the flies have collected at the bottom of the vial, the cotton plug may be removed safely, and the food or moisture added.

For examination, flies are usually first etherized. For this purpose a clean dry vial fitted with a cork and ether pad on a wire is used. First transfer the flies from the culture to this clean, dry, vial, then dip the ether pad into ether, and cork up the vial with the flies in it. Subject the flies to ether for about 30 seconds after they cease moving about, then empty them out onto a clean sheet of white paper for examination; they will remain quiet 4 or 5 minutes. When properly etherized the wings remain in normal position; if overetherized, they stand out above and at right angles to the body. A soft camel's hair brush should be used for handling etherized flies.

G. Isolating Virgin Females.—To obtain virgin females, the culture bottle should be thoroughly emptied of all flies. Six to eight hours later the females which have emerged may then be isolated and used in the matings. Often it is convenient to empty the bottles late in the evening and to take the females out early the next morning. A more accurate method is to place single pupæ in small cork-stoppered vials containing a strip of moistened filter paper or paper towelling. Pupæ which are about to emerge should be selected. The flies in the breeding vial may be transferred to a dry vial temporarily while removing the pupæ. Pupæ are easily transferred by using a dissecting needle, but care must be taken not to injure the pupa when lifting and depositing it.

H. Sex Differences.—The following characteristics may be used in distinguishing the sexes in Drosophila:

(*a*) *Size.*—The female is slightly larger than the male.

(*b*) *Shape.*—The caudal extremity of the male is rather round and blunt while that of the female is sharp and protruding. The abdomen of the male is inclined to be relatively narrow and cylindrical, whereas that of the female is fuller and tends to be spherical.

(*c*) *Color.*—The black marking on the caudal extremity of the male extends around and meets on the under side while that of the female is confined to the top and never meets on the under side. This is the most useful and easily recognized difference in distinguishing sex. The intensity of the black is less in the female than in the male, some of the females being almost devoid of the black pigment. For a few hours after emer-

ging from the pupa case both males and females appear very much alike, but the differences become marked in a short time. In case of doubt, the flies may be kept a day or two, by which time the differences will become more marked. Young flies will live without food (but not without moisture) for about 2 days, after emerging from the pupa cases.

I. Reporting Results.—Careful notes should be kept for each experiment. They should include a clear statement of the cross made, differences between parents, date on which mated, dates of first appearance of larvæ, pupæ, mature flies, etc. The generation should, of course, be noted and particular attention given to segregation in F_2. Each generation should be sorted into the different classes of which it is made up, and the number of individuals of each sex in each class should be noted. At the completion of each experiment there should be prepared a concise statement of the mating, the results in F_1 and F_2, the kind of inheritance illustrated by the experiment, a comparison of theoretical and observed results by the use of the probable error formula or by Harris' method (see p. 100 in text-book), and finally a small chart should be prepared giving an ideal representation of the course of the experiment (see Fig. 1). Outline cuts for use in making charts are furnished to the students.

J. Order of Exercises.—Necessary work with the vinegar flies should take precedence over all other laboratory exercises. Bad conditions of cultures due to neglect of observations between regular laboratory meetings must not be allowed to interfere with other required work. If cultures become contaminated or the insects die, extra time should be devoted to securing a fresh start.

1a. Drosophila Ia.

1. See that you receive a wooden holder with three large and several small vials. Two large vials marked + and *V*, should contain pure strains of wild type and vestigial flies respectively. Do not remove the cotton plug. If you have not already done so, read the general directions for Drosophila experiments. The small vials are for use in isolating virgin females, and the large cork-stoppered vial is for use in etherizing flies.

2. Read general directions for the isolation of virgin females. Transfer all adult flies from the *V* culture to the etherization vial. Then isolate 5 or 6 pupæ from this culture. A few days later when the flies emerge from the pupa cases, mate one or more of the females with + males, and start a culture with them. This culture will be labelled I, and will be the starting point of Drosophila Experiment I.

3. Begin a page of notes on this experiment. At the beginning of your page of notes copy the following:

The plan of this experiment is to cross a *V* female with a + male, removing the parents after eggs have been laid and larvæ are hatching,

and raise the first generation of flies in this same bottle. When several mature flies have emerged, 2 or 3 of each sex will be transferred to another bottle containing food in order to raise the second generation.

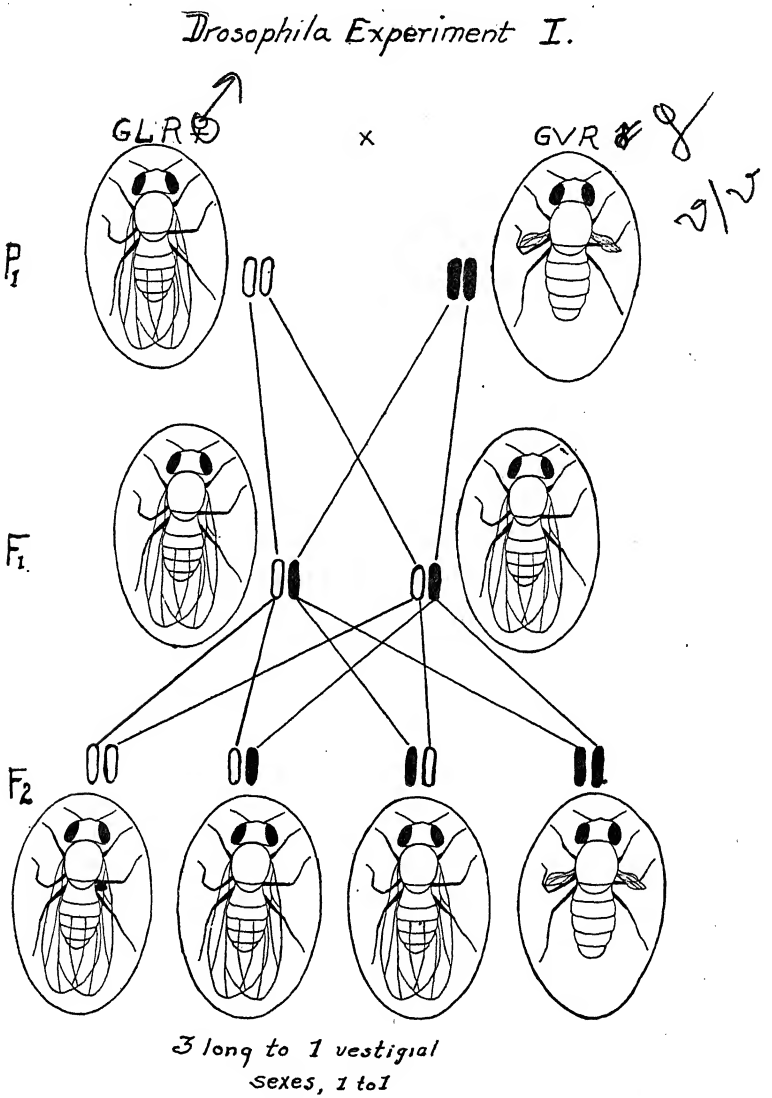


FIG. 1.—Chart submitted by a student as part of his report on a Drosophila experiment.

A week later these parents will be removed from the breeding vial and counted along with the F_1 flies that have emerged from the original bottle. Finally as the second generation (F_2) flies appear, they will be counted and classified according to wing character and sex, and a careful record

kept of the number in each class. From the results secured inferences will be drawn concerning the mode of inheritance of the characters in question.

4. Etherize the + flies in the etherization vial and examine them carefully with a hand lens or dissecting microscope. Make sketches at least 3 inches long of a fly of each sex, showing the dorsal view. Label head, thorax, abdomen, wings, eyes, legs. Below the drawings, note the color of the eyes, and body, making special note of sexual differences in color, and any other difference you see. Similarly remove, examine and make drawings showing ventral view of specimens from the *V* culture. Note particularly in what characters they differ from the wild type.

1b. Drosophila Ib.—Substitute black body color for vestigial wings.

1c. Drosophila Ic.—Substitute sepia eye color for vestigial wings.

2a. Drosophila IIa.

1. If Drosophila Experiment I has not yet been started take steps immediately to get it under way. You should receive an *M* culture either at this period or later in the week for use in Drosophila Experiment II.

2. Drosophila Experiment II. Isolate + virgin females as for Drosophila Experiment I and mate them with *M* males. This experiment is to be conducted in the same manner as the preceding experiment and is designed to illustrate the inheritance of a different wing character. Compare the F_2 distribution of sex with that obtained in Experiment I.

3. The notes for each experiment should be kept separately. The record should be continued to the end of the experiment; *i.e.*, until the F_2 flies have emerged and have been counted and classified. Completeness should be emphasized—all items of any interest whatsoever should be included. The following chronological record should be kept for each experiment:

Date when culture was started.
Date when larvæ first appeared.
Date when parents were removed.
Date when pupæ first appeared.
Date when first mature flies appeared and were transferred to a new vial.
Date when culture was discarded, etc.

2b. Drosophila IIb.—Substitute yellow body color for miniature wings.

2c. Drosophila IIc.—Substitute white eye color for miniature wings.

3a. Drosophila IIIa.

1. Continue work with Drosophila Experiments I and II. The pure strains of + and *V* flies should be retained for they will be needed in subsequent experiments.

2. Pure strains are best maintained by transferring a few flies to fresh bottles of banana as soon as the supply in the old bottle is exhausted. In general cultures need not to be fed more than once.

3. Drosophila Experiment III is designed to illustrate a typical Mendelian dihybrid ratio. For the experiment cross vestigial females with ebony males. The experiment should be conducted in the same way as the first two, but particular pains should be taken to obtain a large F_2 population because of the more complex results obtained.

3b. Drosophila IIIb.—Substitute black body color and sepia eye color for vestigial wings and ebony body color.

3c. Drosophila IIIc.—Use vestigial wings and sepia eye color.

4a. Drosophila IVa.

1. Drosophila Experiments I, II and III should now be under way. If they are not, consult with the instructor in charge as to the best way to deal with them. Make a careful study of the F_1 in each case, noting in what respects they differ from the contrasted characters of the parents. When the F_2 flies have emerged study them in the same manner, classifying them with respect to body characters and sex. It is especially important to note the distribution of sex in each wing class. A successful culture should produce 100 flies or more.

2. Drosophila Experiment IV is designed to illustrate a particular type of dihybridism. For the experiment cross black females with vestigial males or the reciprocal. The work is to be conducted in the same way as other experiments, but more particular pains should be taken to secure a large F_2 population on account of the increased complexity of the experiment.

4b. Drosophila IVb.—Substitute sepia eye color and ebony body color for vestigial wings and black body color.

4c. Drosophila IVc.—Substitute white eye color and miniature wings for vestigial wings and black body color.

5a. Drosophila Va.

1. Continue the work with Drosophila breeding experiments. In case a mating fails to produce larvæ, start it over again without delay. A good way to do this is to obtain a successful pair of flies from some other student who is ready to discard them. Simply transfer them to a fresh vial of banana and they will almost invariably give a successful culture. If a student is getting behind in the experiments he may get F_1 flies from some other student, but in this case he should not neglect to carry out his cultures for an F_1 himself, because he should have a count of that generation and he should make a study of its characters.

2. Students are again reminded to read carefully the general directions for Drosophila breeding experiments. These directions have been planned to give specific directions for carrying out all assigned experiments.

3. Drosophila Experiment V is given to familiarize the student at first hand with the methods and results of studies in inheritance in Drosophila. The problems, methods, results and interpretations have been outlined fully in order to give a definite standard according to which the various Drosophila experiments are to be conducted.

(*a*) *The Problem.*—To determine the mode of inheritance of the yellow body color in a mutant strain of Drosophila when contrasted with the gray color of the normal wild form.

(*b*) *Method of Procedure.*—Reciprocal matings Gray × Yellow and Yellow × Gray are made and the F_1 and the F_2 progenies from the two matings are studied and classified as regards body color and sex.

(*c*) *The Results.*—In the cross $G \times Y$ there is obtained in F_1 a population consisting entirely of gray-bodied flies. The F_2 from such F_1 parents is represented by the population in the vial marked F_2GY, which will be given you. Transfer these flies to the etherization vial, etherize them thoroughly, then segregate them into classes as regards body color and in respect to sex. Note that the sexes are represented in approximately equal numbers, and that all the females have gray body color and that about one-half of the males or one-fourth of the F_2 have yellow body color.

Vial F_2YG contains the F_2 progeny from the reciprocal cross, Yellow female × Gray male. Examine this population in the same way. The F_1 in this case consisted of gray females and yellow males. How does the F_2 ratio of this mating compare with that of the gray × yellow mating?

(*d*) *The Interpretation.*—The explanation of these results rests on two facts indicated by the experimental evidence; first that the cross deals with a single pair of contrasted characters as indicated by the 3:1 ratio in F_2, and second that the factors representing these characters are sex-linked, as indicated by the fact that one-half of the F_2 males were yellow in the first cross, $G \times Y$, and by the criss-cross inheritance in the F_1 population of the reciprocal cross. Representing the yellow factor by y, since it is recessive as shown by the gray color in the F_1 of the cross gray × yellow, and the dominant allelomorph conditioning gray body color by Y, and representing the sex-chromosomes by X and Y, we have the hereditary formula for the parent individuals $(YX)(YX)$ = gray females; $(YX)Y$ = gray males, $(yX)(yX)$ = yellow females and $(yX)Y$ = yellow males.

(*e*) The theoretical consequences of these assumptions are outlined below. The factor for yellow, as well as its dominant allelomorph, is carried by the X- or sex-chromosome which is indicated diagrammatically by enclosing in parentheses the factor symbol, y or Y, with the X. The maturation of the germ cells results in the formation of gametes having one-half the somatic number of chromosomes. Thus each egg has one

X-chromosome which carries y, the factor for yellow, when a yellow female is concerned or the dominant allelomorph Y, if from a gray fly. The same mechanism results in the formation of two unlike classes of sperm, one-half containing X with the sex-linked factors and the other half containing a Y which is not known to carry any character factors. In the cross $(YX)(YX) \times (yX)Y$ the F_1 are all gray due to the fact that every daughter received one (YX) from the mother which determines the phenotypic expression, while the (yX) from the father is carried unexpressed phenotypically, although such flies are true hybrids. The sons $(YX)Y$ receive their only (YX) from their mother which determines their gray color, and a Y-chromosome from their father. They are pure gray flies, *not* hybrid for the yellow color. The F_1 females produce two kinds of eggs (YX) and (yX); the F_1 males produce two classes of sperm (YX) and Y, but only one (YX) is concerned with the sex-linked factor for body color. When these F_1 individuals are mated the gametes pair according to the law of chance and the following combinations result.

Zygotic constitution of F_2 =	$(YX)(YX)$,	$(YX)(yX)$,	$(YX)Y$,	$(yX)Y$
Phenotypic expression of F_2 =	gray females	gray hybrid females	gray males	yellow males
Phenotypic ratio	= 3 gray to 1 yellow			
Sex-ratio	= 2 females to 2 males or 1:1			

In F_2 all the females and one-half the males have the normal gray color.

In the cross yellow female (yX) $(yX) \times$ gray male $(YX)Y$ the same distribution of sex-chromosomes occurs but with different results regarding the sex-linked factor. The F_1 males receive their only (yX) chromosome from the mother and are as a consequence yellow of a pure zygotic constitution. The daughters receive one (YX) from the father and are pure gray in color although hybrid for the yellow factor. These females are of the same factorial composition as the F_1 females of the reciprocal cross and therefore produce the same kind of eggs. The F_1 males being pure for the yellow factor can produce only yellow-bearing and neutral sperm. The mating of these F_1 flies results in the following F_2 combinations.

Zygotic constitution =	$(YX)(yX)$,	$(yX)(yX)$,	$(YX)Y$,	$(yX)Y$
Phenotypic expression =	gray hybrid females	yellow females	gray males	yellow males
Phenotypic ratio	= 2 gray to 2 yellow or 1:1.			
Sex-ratio	= 1 gray female : 1 gray male : 1 yellow female : 1 yellow male.			

In this F_2 population one-half the flies of both sexes are yellow.

(*f*) Prepare charts for the notebook to illustrate the ideal course of events in these two crosses. Each chart should be allowed a full page and the figures should be so arranged as to illustrate the problem neatly and clearly (see Fig. 1).

5b. Drosophila Vb.—Substitute white eye color for yellow body color.

5c. Drosophila Vc.—Substitute miniature wings for yellow body color.

6a. Drosophila VIa.

1. Each Drosophila experiment is to be reported complete in itself. Reports may be handed in as soon as the entire F_2 population has been classified. The larger the F_2 population the nearer the expected results will be realized. Each report should contain a chronological record, sex and character data of the F_1 and F_2 populations, the number of flies, the observed ratio per four, the deviation, the probable error (see p. 100 in text-book) and the probable chances of the occurrence of the observed deviation (from Pearl's table; see Table IX in Appendix I) also a written explanation of the results together with an illustrative chart (see Fig. 1).

2. Drosophila Experiment VI is the last assigned experiment with Drosophila. It is a problem in the creation of new varieties. Starting with pure strains of black and vestigial flies, the task of the experiment is to produce a strain of black vestigial flies. Determine accurately what sort of matings are necessary to produce such results. Keep full records of each mating. Some of the material from Experiment IV may be used for this experiment. The black vestigial flies are to be turned in with the report.

To assist the student in planning his procedure in this experiment we give below a brief description of Morgan's method of determining the group to which a new mutant character belongs.

The Character Groups in Drosophila melanogaster (ampelophila)

By means of experimental breeding the inheritance of over 150 distinct characters in this species has been worked out. It has been found that these characters belong in four groups. How has this analysis been made? By studying the characters as they appeared. For example, when the first white-eyed male was found it was crossed with a red-eyed female. The F_1 flies were all red-eyed and when inbred they produced in F_2 3 red-eyed flies to 1 white-eyed fly, but the white-eyed flies were all males. Soon other characters were found that were inherited in the same way as white eyes and thus the first or sex-linked group of characters was distinguished. At the same time characters were observed that were not sex-linked and by studying the association of these characters one with another in a vast number of breeding experiments the second and third groups were distinguished from each other and finally two characters were found which were not associated in inheritance with any one of the first three groups and which must therefore belong in a group by themselves.

The usual procedure for locating a new mutant character, which may be represented by x, has been outlined by Morgan as follows:

If x does not show sex-linked inheritance its chromosome is determined by taking advantage of the fact that in Drosophila there is no crossing-over in the male between factors in the same chromosome. The following tests are used: It is crossed to black, whose factor is known to be in the II chromosome and to pink whose factor is known to be in the III chromosome. If the factor for x should happen to be in the II chromosome, then in the cross with black no double recessive can appear, so that the F_2 proportion is 2 : 1 : 1 : 0 but with pink x should give the proportion 9 : 3 : 3 : 1, typical of free assortment.

If, however, the factor for x is in the III chromosome, then when crossed to black the double recessive and the 9 : 3 : 3 : 1 proportion appears in F_2. But when crossed to pink no double recessive appears in F_2 and the proportion 2 : 1 : 1 : 0 occurs.

If these tests show that x does not lie in either the II or III chromosome, that is, if both with black and with pink the 9 : 3 : 3 : 1 ratio is obtained, then by exclusion the factor lies in the IV chromosome, in which as yet only two factors have been found.

It is obvious that these two tests can be made by a single cross between black pink flies and flies pure for x. If in F_2 some bx but no px flies are found it is evident that x is not in the II chromosome but is in the III chromosome. Conversely if some px but no bx flies are obtained in F_2 then it is clear that x is in the II chromosome. But if both bx and px flies appear in F_2 it follows that x must lie in the IV chromosome. This must be tested by crossing x with eyeless or bent. As a matter of fact Morgan uses a strain of black bent pink flies in making his first test of x. Having located x in its group it only remains to test its linkage with two or three factors, whose relative locations in the same chromosome have been determined, in order to predict its linkage relations with all the other known factors in that chromosome (consult Chapter VI in text).

A clear understanding of the above-described method will be of value in connection with the creation of new varieties of *Drosophila melanogaster* (*ampelophila*) by means of crossing existing strains. Such new varieties are new only in the sense that they possess combinations of characters which are different from previously existing combinations. If the characters involved have been located in their groups and the linkage relations between the factors determined, the types of matings necessary to produce a pure strain of the desired new combinations and the approximate number of matings which it will be necessary to make can be worked out. This should be done in the case of Experiment VI before mating any F_1 flies.

6b. Drosophila VIb.—Substitute ebony body color and sepia eye color for black body color and vestigial wings.

6c. Drosophila VIc.—Substitute miniature wings and white eye color for vestigial wings and black body color.

II. VARIATION IN PLANTS

7a. Variation in a Population of Broad or Windsor Beans.

1. With a small metric rule measure the extreme length and width of about 200 broad beans, recording measurements to the nearest millimeter. Record the number of the lot measured. Record each measurement as it is taken using coördinate paper and arranging three columns headed *No. of Bean, Length, Width.*

2. From the data thus obtained construct a correlation table. Use squared coördinate paper and write the widths at the top and the lengths along the left side of the correlation table. Then fill in the data by the simple method of tallying, making a mark in the appropriate square for each bean. When this has been done, obtain the totals for each square and transfer the data thus obtained to a new correlation table. From this table the frequency distribution for length may be obtained by taking the sums of the beans in the rows corresponding to given lengths; for width by taking the sums of the beans in columns corresponding to given widths.

3. When the data have been arranged in frequency distributions, construct graphs showing the frequency distributions for length and width of the beans that have been measured. Indicate the position of the mode in each graph by a perpendicular straight line. These graphs should be constructed roughly during the laboratory period, and may be finished in detail at home. Make the graphs on such a scale that each one occupies the major portion of a page. From the observed distributions what inferences may be drawn as to the general nature of variation in such populations?

4. Students may work in pairs for this exercise. *The data, however, should all be in each student's notebook;* it may be used later in the study of biometry. (NOTE.—No further reference is made to the use of these data. It is left for the instructor to make such use of this material in connection with Chapter III of the text-book as he sees fit.)

7b. Variation in a Population of Maize (the Ear).—Method as in **7a.** Use about 200 ears of any variety of maize, measuring length and circumference to the nearest centimeter with a steel tape. If there is sufficient time for taking more data, each ear may be weighed after it is measured, the rows may be counted, the ears shelled and grains measured, etc.; thus providing data for considerable practice in biometry. It will be found convenient to number the ears using paper string tags or small tree labels fastening with wire.

7c. Variation in the Garden Pea.—Biometrical problems.

(*a*) From the data in Tables I and II find the mode and mean for each of the three variable characters. Plot the curves of the three variables. Explain the difference of mean from the data of the two plots.

(*b*) Make a correlation table for Number of Peas and Weight of Peas, using the following indicated classes.

Divide the variables into the following classes:

Height of plants

13.1–17	$V = 15$
17.1–21	$V = 19$, etc.

Number of peas

1 and 2	$V = 1.5$
3 and 4	$V = 3.5$, etc.

Weight of peas

0–1000 mg.	$V = 500$ mg.
1001–2000 mg.	$V = 1500$ mg., etc.

TABLE I.—DATA ON *Pisum sativum*, THE GARDEN PEA

Plot 1 (*Highly Fertilized Soil*)

Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.
43.0	10	2075	52.5	34	8375	25.0	6	1540	38.0	6	1585
33.5	18	4935	44.0	11	2750	38.8	11	2400	53.0	27	4965
43.0	27	7610	39.0	15	3695	46.5	15	3625	43.0	30	6420
41.0	12	2525	57.5	31	7480	56.5	13	2290	60.0	30	6145
46.5	14	3030	58.0	21	4645	49.0	18	5760	52.5	20	4430
38.0	13	2530	42.5	21	3800	36.0	5	1345	61.0	22	5345
32.0	3	640	40.0	8	1500	35.5	6	1545	25.0	5	900
39.0	9	2685	79.8	28	5220	65.0	15	3255	57.0	15	2975
52.0	13	1795	64.0	21	4450	44.5	19	4255	67.0	44	9185
35.0	6	1060	49.0	22	4580	71.0	20	4025	45.5	15	3565
41.3	11	1825	28.5	4	675	47.3	12	2295	43.5	13	3095
37.0	9	1965	48.0	7	1065	33.0	6	1565	35.6	13	3130
44.0	12	2810	41.5	13	2500	57.5	33	5840	36.0	6	1030
61.0	30	6590	65.0	19	3890	42.0	9	1765	56.0	17	3635
46.0	34	6420	55.0	24	4890	50.5	13	2760	44.0	10	2135
50.0	15	2360	26.5	16	1220	41.0	26	5960	60.0	28	6620
39.0	12	2360	45.0	11	2610	61.0	9	2050	53.0	15	3270
57.0	30	6870	36.0	10	2310	49.0	15	3685	29.5	6	1765
48.0	22	5125	55.0	16	3455	46.5	18	4345	61.0	21	4220
47.0	23	4815	48.0	13	1875	52.0	22	4225	42.0	8	2365
42.0	8	2070	47.0	19	4095	53.0	25	5535	46.0	15	3060
40.7	14	3050	48.0	8	1635	51.5	12	2125	45.0	10	2730
47.0	16	3535	48.0	13	2295	57.0	23	3755	36.0	14	3020
46.5	18	4235	28.5	3	585	35.0	12	3115	50.0	15	3915
44.0	15	3595	49.0	13	2950	38.0	15	3865	45.0	17	4110
34.3	7	1500	48.5	11	2450	44.0	7	1445	42.0	6	1675
28.5	2	575	47.0	14	2470	34.0	3	855	53.0	17	4150
57.0	20	3885	61.5	31	6335	48.0	12	2740	57.5	13	3325
60.0	19	2655	50.0	16	3215	61.7	20	4745	49.5	11	1800
50.0	19	3735	36.0	13	3325	54.5	17	3325	48.5	16	2640
51.0	15	2915	41.8	16	3360	44.5	15	3630	39.0	8	1820
47.0	16	4025	45.0	8	1800	55.0	23	5090	58.5	22	4370
48.0	11	2535	39.0	10	2260	39.0	10	2915	39.0	13	2985
36.0	12	3155	39.0	25	5950	47.5	17	3860	44.5	19	3475
32.0	5	540	36.5	10	2560	45.0	12	2315	43.0	10	3155
62.0	22	4955	56.0	9	1400	46.0	40	9585	66.0	29	4855
51.0	28	8015	35.0	8	1745	61.0	15	4385	47.0	12	3070
57.5	25	6070	51.5	11	2500	42.5	11	1835	44.5	13	2895
62.0	30	7960	45.0	10	2220	51.0	26	5650	41.0	8	1050
47.0	11	2745	47.0	14	3525	46.0	8	1140	65.3	17	3515
57.0	20	5030	49.0	11	2550	77.0	7	1690	46.0	18	4880
46.5	7	1685	43.4	18	4660	55.5	22	4320	65.0	14	2755
51.0	11	2450	52.7	21	4335	46.7	5	1070	46.5	5	470
51.0	11	2450	51.0	24	4395	55.0	39	8400	49.5	7	1515
61.0	40	8570	47.0	15	3120	37.0	28	5725	52.0	21	4395
52.0	15	3050	47.0	7	1170	41.0	15	3295	50.5	21	3990
33.0	5	1280	61.5	17	3705	53.0	41	9790	59.0	31	6670
57.0	23	4795	55.0	15	2885	64.0	20	4410	28.5	1	70
40.7	7	1820	39.5	9	2275	47.0	10	2450	53.0	19	2635
67.0	24	4490	54.5	12	2735	63.0	42	10075	48.0	8	1110
61.0	14	2910	52.0	17	4515	54.5	24	6085	49.0	31	6670
42.0	11	2085	42.0	9	2465	47.0	15	3480	53.0	13	3090
55.5	24	3595	62.0	35	7380	63.0	11	2300	70.0	24	4570
39.0	13	1780	63.0	7	1825	67.5	18	3320	57.5	2	460
58.0	20	4540	64.0	31	6555	52.3	21	2930	50.0	4	290
57.7	31	6345	52.5	9	1440	67.5	17	2755	55.7	30	5995
60.0	26	6750	61.0	19	4080	47.0	8	773	25.0	3	660
57.0	25	6110	55.0	21	6190	56.0	13	2460	51.5	22	6205

TABLE II.—DATA ON *Pisum sativum*, THE GARDEN PEA

Plot 2 (*Ordinary Soil*)

Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.	Height	No. of peas	Wt. of peas mg.
30.0	6	1490	38.5	12	2885	32.8	7	1945	35.0	6	1575
30.5	8	1770	27.0	5	820	35.3	8	2135	55.0	16	3810
29.8	8	2020	35.0	3	760	29.5	9	2505	48.5	13	3355
40.5	9	2195	39.0	6	1280	42.0	10	2690	55.5	14	3500
27.0	6	1645	36.0	9	2325	28.8	7	1670	45.0	16	3425
36.5	11	2435	30.5	8	1300	40.9	10	2660	32.7	9	1765
34.5	10	2625	46.0	17	3560	32.7	7	2000	43.3	10	2400
20.5	2	555	30.5	8	2160	28.4	1	90	45.5	16	4210
30.0	6	1445	41.0	10	2140	43.5	11	2465	29.5	3	875
38.5	12	2740	16.5	2	325	29.0	6	1600	47.0	12	2750
39.0	9	2470	30.0	11	2860	40.0	9	2415	27.7	5	1365
45.0	12	2960	34.8	6	1570	33.9	8	2055	27.5	7	1790
26.4	3	865	32.3	9	2115	33.5	8	1745	41.5	10	2695
32.0	6	1230	36.6	11	2335	34.5	6	1210	52.5	13	2980
35.2	10	2985	30.0	4	630	26.5	4	1180	36.5	21	4055
44.5	11	2690	37.8	6	1090	24.5	4	930	27.5	6	1555
41.2	13	2965	44.0	6	1130	53.0	19	4925	50.0	12	2375
41.3	12	3090	39.5	9	1860	31.7	7	1810	39.0	9	2455
40.0	11	2205	37.0	6	1655	44.5	12	3545	28.5	6	1470
28.0	7	1755	36.5	10	2160	54.0	21	5025	28.5	7	1410
30.5	6	1360	36.0	9	1875	48.5	17	3245	33.0	8	2335
29.0	5	1445	33.3	7	1765	39.0	11	2515	59.0	23	4630
54.8	14	3295	27.5	7	1660	40.5	10	2440	27.0	5	975
47.5	15	3585	49.5	11	2745	47.0	10	2160	38.5	9	2075
26.3	5	1180	45.0	13	2990	42.0	11	2030	57.0	19	3490
42.0	12	2700	31.0	5	870	41.3	6	1270	72.0	25	5625
36.0	9	2100	35.4	6	1820	42.1	10	2580	29.3	5	985
27.0	7	1275	42.3	11	2755	24.5	4	925	23.5	5	715
30.0	8	1690	26.3	5	1205	46.5	16	3405	43.0	13	2760
45.0	12	2630	40.5	10	2430	53.0	16	3650	51.5	18	2960
42.0	7	1785	51.7	13	2210	45.0	7	1640	67.0	12	2560
39.0	11	2595	51.0	11	1905	50.0	21	2430	39.6	8	2050
28.0	5	1420	51.0	10	1915	44.5	12	2885	45.0	15	3065
51.0	15	3305	58.0	8	2400	44.6	15	4065	34.0	13	2190
38.0	9	2040	38.0	6	1570	42.0	12	2385	33.5	7	1355
46.0	9	1210	34.5	6	1590	48.0	14	2875	45.0	9	1870
48.5	13	2870	53.5	30	5235	40.5	14	3305	51.1	13	2790
50.0	12	2930	55.0	12	2750	43.0	9	2485	59.5	16	3920
42.0	12	2845	58.0	12	2810	24.5	5	1225	53.0	10	1660
28.5	6	1545	65.6	28	4945	48.8	15	3795	50.5	8	1455
46.5	14	3475	35.0	6	1490	36.0	12	3135	43.0	10	2390
54.5	6	355	37.5	8	1900	46.5	23	4270	37.5	7	1455
39.0	11	2785	53.0	19	4155	26.5	6	1585	33.3	5	1140
47.8	11	2680	43.5	8	2000	49.0	10	3095	53.0	25	4595
61.5	33	6880	35.5	6	1805	61.0	17	2965	63.0	22	5445
35.0	6	1690	38.3	7	1985	50.0	30	6430	75.3	15	2900
40.5	18	3670	29.0	4	995	32.0	5	1140	56.0	13	2500
39.7	11	1635	46.3	12	2900	37.0	9	2070	42.0	6	1315
42.5	18	4545	36.0	6	1320	58.5	11	2380	55.0	12	1800
48.0	9	2125	47.0	10	2180	47.0	16	3580	28.5	5	700
64.0	6	1830	39.0	9	1760	21.0	5	1140	47.0	8	1470
38.5	12	2540	29.0	6	1425	46.0	8	1835	47.0	6	1600
43.0	15	2865	48.0	10	2495	56.0	9	2125	53.0	11	1730
46.0	13	3400	50.0	13	3375	42.5	8	1895	56.0	11	2435
52.0	12	2610	32.3	7	1520	42.5	18	4220	52.0	9	2540
33.0	7	1505	48.0	16	3725	38.0	8	2250	26.5	4	1065
41.0	10	2080	27.5	4	1145	35.5	7	1350	46.0	5	1190
22.5	2	535	33.0	7	1705	41.0	6	1450	83.0	23	4845
32.0	2	625	34.0	7	1570	52.5	12	2830	40.0	5	1410
42.5	7	1655	29.0	6	1490	51.6	21	4620	63.0	14	3015
20.0	2	505	40.0	11	2475	55.0	25	5245	46.0	7	2055
44.0	17	4475	52.0	15	3565	48.0	17	3615	31.0	6	1310
36.0	5	1355	47.7	20	4265	44.3	13	2640	46.0	20	4215
42.0	11	2750	31.0	5	1155	29.0	9	1935	41.0	18	3720
40.0	7	1410	53.4	24	5240	37.8	15	2385	54.0	14	2195
45.0	1	235	37.0	8	2265	47.0	18	4155	22.5	3	645

8a. Variation in Stellaria media, Common Chickweed.—Field study.

1. Study variation in *Stellaria media*, the common chickweed, according to the plan outlined below. Tabulate data wherever possible, and prepare a special report of the work done with this plant.

2. *Description.*—From notes taken in the field prepare a description of this species giving attention to the following features: Habit and general appearance of plant; leaves—shape, variation in shape on the same stem, presence or absence of petioles on same stem; flowers—color, where borne on plant, position of pedicel in flower and in fruit; sepals—number, hairy or glabrous; petals—number, length compared with sepals; stamens—number; pistil—simple or compound; styles—number; ovary—shape, number of cells; fruit—a capsule with how many valves, how dehiscent? Examine entire plants for pubescence and state what you find.

3. *Data on Number of Stamens and Pubescence.*—Examine at least 100 different flowers, either in the field or in material gathered at specified locations. That is, if you prefer to examine your flowers in the laboratory or at home, keep samples from different populations separate. In counting stamens examine material from at least two different locations and in case you find that the mode differs then calculate the constants and prepare the graphs called for (see Par. 5) for each population. Do not lump material from different locations in this work. (1) Count and record the stamens. (2) Note presence or absence of pubescence on sepals. (3) Note presence or absence of pubescent line on stem and pedicel, also any variations in same.

4. Does the character of hairiness or smoothness of sepals vary on the same plant as to presence or absence or only between different plants? What do you conclude as to the way in which these characters are inherited? What proportion of the plants examined have smooth sepals?

5. Tally stamen counts in classes from 1 to 10 or more including each integer. Calculate mean, standard deviation, and coefficient of variability for number of stamens. Draw a frequency polygon to show variation in number of stamens.

8b. Variation in a Common Wild Plant Species Exhibiting Several Distinct Types.—Laboratory and field study.

1. Study carefully 3 to 5 forms in the laboratory making for each outline drawings of typical leaves, inflorescence, etc. Have all the forms before you at the same time, and make note of differences especially those which cannot be shown in drawings. In describing quantitative features report actual measurements.

2. In the field, if possible, make notes on a few populations for the purpose of determining the nature of their variable characters, whether germinal or merely somatic; whether heritable or environic. What sort of variation do you find within populations, merely in the degree of expression of a given set of characters or in actual differences of characters? Do you find conspicuous variations of given characters within a single plant? Do you find populations in different stations which are alike in all characters? Do you find evidences of hybridization in cases where

the stations of two different forms overlap? What should you consider evidences of hybridization? What do you think accounts for the variety of forms which this species displays?

8c. Herbarium of Variations.—General directions.

Object.—To broaden the student's acquaintance with variations in plants and encourage observation of the same.

Materials.—Collecting outfits will be furnished by students. Before putting in press see that each sheet bears the data necessary for writing a label. Do not remove from press until thoroughly dry. Directions for mounting are given below. Search for 5 specimens each of morphological and meristic, and 4 each of substantive and functional variation and one of place variation; also plan one sheet of morphological and one of meristic variation so as to show fluctuating variation.

Collecting.—Take as nearly the whole plant as is practicable. The size of the mounting sheets is 11 by 16 inches. When you collect your specimens plan with reference to this size of sheet and arrange them accordingly when you are putting them into the driers. Do not put large woody branches containing thorns, galls, knots, or other irregularities into press with other specimens. Use special care to press leaves and flowers flat. Do not try to lift the whole plant from specimen sheet until thoroughly dry. *Always* collect flowers and fruits if possible.

Mounting.—In regular herbarium practice specimens are mounted by floating them on a dilute solution of glue and then laying them carefully on the mounting paper. This requires practice. Most specimens can be satisfactorily mounted by placing minute drops of dilute glue on the intended reverse side of the specimen and then putting it under pressure after carefully arranging it on mounting paper. After 24 hours carefully examine and place more glue under parts that are loose. *Specimens must be thoroughly dry before mounting.*

Labeling.—For labeling specimens blank slips of paper will be furnished. Fill out each slip in following order:

1. Kind of variation.
2. Scientific name of plant.
3. Common name of plant.
4. Locality.
5. Date collected.
6. Collector's name.
7. Explanatory notes.

Attach label on *lower right corner*, leaving a small margin. Shorter dimensions of mounting sheet are considered as top and bottom. Attach the label with a little library paste at the corners *only*. *Do not* cover the back of the label with glue or paste. Neatness is absolutely essential.

9a, b, c. Bud Mutations in the Boston Fern, *Nephrolepis exaltata bostoniensis.*

1. This fern is notable for the great variety and diversity of form of its bud mutations. It is believed to have arisen from *N. exaltata,* but the exact manner of its origin is not clear. *N. exaltata* can be readily propagated from spores, but Bostoniensis is propagated with great difficulty in this manner and however carefully the spores are treated only a small percentage of them germinate. Possibly it is a hybrid.

2. The following two series of forms will be given for study; first, one which shows progressive changes in the size of the frond; and second, a series of plumose forms which is based on increasing complexity in the dissection of the pinnæ. A pinna is one of the main divisions of a frond; a pinnule is a subdivision of a pinna. (Other varieties may be substituted. The nomenclature used below is horticultural not botanical. In series II Nos. 1 and 2 are included because they represent the starting point of the entire group of dissected varieties.)

Series I.—Size of Frond

1. *N. Exaltata*
2. *N. Bostoniensis*
3. *N. Giatrasi*
4. *N. Roosevelti*
5. *N. Harrisi*
6. *N. Washingtoniensis*
7. *N. Whittboldi*
8. *N. Robusta*
9. *N. Springfield*
10. *N. Teddy, Jr.*

Series II.—Dissected Pinnæ

1. *N. Exaltata*
2. *N. Bostoniensis*
3. *N. Piersoni improved*
4. *N. Robusta*
5. *N. Scholzeli*
6. *N. Amerpohli*
7. *N. Exaltata plumosa*
8. *N. Muscosa*
9. *N. Smithi*
10. *N. Whitmani*

3. Starting with a typical frond of Bostoniensis, make an outline sketch to scale which will show the relative length and width of frond, and then draw a pinna in detail. Make similar drawings of at least four other members of each series. All drawings should be made to the same scale, and the scale used should be indicated at the bottom of the page. A scale of about one-fourth will be found convenient to use. In each case draw pinna life-sized.

4. Discuss variations in Nephrolepis as illustrated by the two series given you. Assuming that the germinal elements in the genus have a structural organization comparable to that in Drosophila, what sort of conception would appear most probable to you as to the relation of the different forms to one another? If they were able to produce spores, would those from each different plant breed true? As to the source of variation, would the variation be somatic or germinal? Sometimes the variations, instead of involving a bud of a runner, may involve only a single frond or only a portion of a frond. In such cases the variation cannot ordinarily be propagated. Would such variations be somatic or

germinal? Can you think of any reason why the forms should group themselves naturally into related series?

5. The genus Nephrolepis, according to Boshnakian, may be divided into two groups; *A*, those which produce new forms only when propagated sexually; and *B*, those which "sport" in asexual propagation. Bostoniensis belongs to the latter group. The members of group *B* bear few spores, sometimes none at all, and the spores are always very slow and uneven in germination. This fact leads to the assumption that the Boston fern is hybrid in nature. Species crosses in tobacco are known to produce results somewhat similar. The Washington navel orange and the evening primrose, *Œnothera lamarckiana,* are other examples of plants giving similar results and which have been accused of being hybrids. Assuming the Boston fern to be a hybrid, can you think of any reason why such hybrids should give asexual variations more abundantly than members of group *A*?

6. A number of varieties of the Boston fern are illustrated in a paper by Barron, "An Interesting Family of House Ferns," *Garden Magazine,* vol. xiv, pp. 261–263, January, 1912. Practical breeding problems are discussed by Boshnakian, "Breeding Nephrolepis Ferns," *Journal of Heredity,* vol. vii, pp. 225–236. Discussions of bud variation in other plants, particularly Citrus and Coleus, will also be found of interest.

10a, b, c. Chimeras and Graft-hybrids.

1. Variation within the individual plant is sometimes due to genotypic heterogeneity of its tissue elements. This heterogeneity is almost, if not quite always simple, that is only two kinds of genotypically diverse tissues occur in the same plant, and these diverse elements usually occupy distinct regions or sections in the plants. Plants which possess genotypically diverse elements are called chimeras.

2. Many variegated plants are chimeras. In them the two genotypically diverse elements are (1) the normal elements which produce chlorophyll in those tissues which normally should produce chlorophyll; and (2) an element which has lost the power to produce chlorophyll, and which consequently produces tissue lacking the normal green color. The mixture of these two elements gives the typical variegated foliage of certain kinds of ornamental plants.

3. Study material of *Sambucus nigra variegata, Deeringia baccata* (*celeosioides*) or a similar variegated ornamental shrub. The plant as a whole is a periclinal chimera with an inner cylinder of normal tissue surrounded by an outer layer of cells not producing chlorophyll. Draw several different leaves showing the relations and relative extent of the different elements. How do the partly green leaves prove that the plant is a periclinal chimera? Be sure you understand perfectly the way in which leaves originate before attempting to answer this question.

4. Such a plant frequently produces sectorial chimeras. Study branches which illustrate this type of chimera. Describe the branches as a whole, noting especially the appearance of the main stem, relation of the color of the stem to the color of the twigs, and relation of the color of the twigs to the color of the leaves. Draw a cross-section of a stem showing the two kinds of tissue, and characteristic twigs in the proper relation to them. Colored crayons will help in making these drawings.

5. Buds which arise from mutilated graft unions sometimes are of the structure of chimeras. Examine plants of *Lycopersicum esculentum*, the tomato, and *Solanum nigrum*, the nightshade, and illustrations of chimeras that were produced from graft unions between these two species. The seeds from these chimeras produce nothing but tomato or nightshade plants. Why is this so? Explain how chimeras may arise from graft unions. Examine material which illustrates how the grafting is done.

6. Sectorial chimeras in oranges, lemons, apples, pears, tomatoes or flowers. (It is difficult to preserve fruits and flowers in a condition to show natural chimeras satisfactorily for class use. Wax models of fruits have been found very useful and, although the initial cost is rather high, they will last indefinitely if handled carefully.) Draw at least one specimen. Explain how such chimeras may arise.

7. Copy the tissue sections from the chart, showing the chimera *Cratægomespilus Asniersii* and its parents. What sort of chimera is it? See also *Cytisus Adami* and its parents. (Both illustrated in Baur, "Vererbungslehre," 2nd edition, pp. 258 and 260.)

III. MENDELISM IN PLANTS

11a. Mendelism in Maize—the Grain. I.

1. The corn grain is made up of several different parts, the diverse behavior of which must be considered in studies of heredity. Grossly, we may distinguish the germ or embryo, and surrounding it the endosperm. The endosperm in a starchy variety is composed of a white floury portion, often called the starchy endosperm, a hard translucent portion called the horny or corneous endosperm, and finally surrounding these two a sheath of cells called the aleurone layer. The entire grain is surrounded by a tough skin, the pericarp, which may easily be separated from the rest of the grain after soaking it in water for a few minutes. Of these tissues the pericarp is maternal and consequently it is not subject to xenia effects. Xenia is the name applied to the immediately visible effects of foreign pollen on tissues of the ovary other than the embryo and is usually restricted to the endosperm. This phenomenon is due to "double fertilization." One of the two generative nuclei of the pollen tube unites with the true egg nucleus of the egg sac thus producing a hybrid embryo, while the other pollen nucleus fuses with the endosperm nucleus causing a "hybrid" condition of the stored food material of the

endosperm which at once becomes visible if the male parent carries the dominant character. The following law regarding xenia has been formulated by East: "When two races differ in a single visible endosperm character in which dominance is complete xenia occurs only when the dominant parent is the male; when they differ in a single endosperm character in which dominance is incomplete or in two characters both of which are necessary for the development of the visible difference, xenia occurs when either is the male."

2. The character distinctions which may occur in the various portions of the grain are as follows:

Corneous and starchy endosperm textures:

Starchy, waxy or sweet.

Corneous endosperm colors:

Yellow of various shades from deep amber to pale sulfur yellow or white.

Aleurone colors:

Purple and red of a variety of shades or blotching or spotting with these or colorless.

Pericarp colors:

Brown and red of a great number of shades, stripings and blotchings of these or colorless.

3. Dissect a number of the different types of corn grains which have been soaked in water. Determine the relations of the various parts to each other and the color character exhibited. Tabulate the results of your study in the form shown in Table III.

TABLE III.—COLOR CHARACTERS IN MAIZE

External appearance of grain	Location of color zones		
	Pericarp	Aleurone	Corneous endosperm
Reddish yellow			
Yellow			
Reds			
Blues			
Black			
Green			
White			
Drab			
Red striped			
Light yellow spotted (white with red or purple)			

4. Draw diagrammatically a typical dent grain in longitudinal section, labeling all the parts. Make drawing about 4 inches long. Why

do not corns exhibit xenia when the female parent plant is from a strain of red corn?

5. Mendelian experiments with maize. In conducting Mendelian investigations with maize, it is necessary to bag each ear before the silks appear, and to hand pollinate with guarded pollen when the silks are receptive. Each envelope given you contains the grains from a single ear which was obtained in this manner, and it, therefore represents valuable material obtained at a considerable expense. Accordingly take the utmost care not to lose any of the grains or to mix those from different envelopes. Work with only one ear at a time. Copy the envelope number in each case and as soon as one lot is counted return the grains to the envelope before opening another. Tabulate the data obtained and compare with expected results for each ear and for the totals of all the ears counted. Calculate the observed ratios and test goodness of fit by computing deviation from expected ratio and the probable error (see p. 100 in text-book). Obtain odds against occurrence of such ratios from Table IX, Appendix I.

(NOTE.—If it is impossible to furnish the class with hybrid maize grains showing segregation of starchy and sugary endosperm, purple and white aleurone, or some other single pair of Mendelizing characters, the data given in Table IV may be used. Let each student study the counts of three or four ears criticizing each ratio as well as the totals of all the ears recorded in Table IV, according to the directions in the preceding paragraph.)

6. Construct an F_2 checkerboard for a cross involving one pair of Mendelizing characters, indicating in each square the F_3 phenotypic ratio that each F_2 genotype will produce if self-fertilized.

7. Varieties of *Zea mays* are for convenience separated into several groups as follows:

1. *Zea mays tunicata*, the pod corns.
2. *Zea mays everta*, the pop corns.
3. *Zea mays indurata*, the flint corns.
4. *Zea mays indentata*, the dent corns.
5. *Zea mays amylacea*, the soft or flour corns.
6. *Zea mays saccharata*, the sweet corns.
7. *Zea ramosa*, branched corn.
8. *Zea japonica*, striped corn.

These groups hybridize and furnish excellent material for the study of heredity. Examine laboratory specimens of each of these groups.

8. References: (1) Inheritance of Waxy Endosperm in Hybrid Sweet Corn, U. S. D. A., B. P. I. *Cir.* 120 (1913).

(2) Dominance of Characters in Corn, *Rept.* Conn. A. E. S., 1908, p. 410.

(3) Xenia, or the Immediate Effects of Pollen in Maize, *Bull.* 22, Division Veg. Path. and Phys., U. S. D. A., 1900.

(4) Inheritance in Maize; East and Hayes, Conn. A. E. S. *Bull.* 167 (1911).

(5) Chapters V, VII, VIII in the text-book.

11b and c. Mendelism in Other Plants. I.

Excellent material for the study of the monohybrid can be secured by the students themselves from the results of Exercise 16 provided this exercise is introduced early in the course. For example the Jimson weed may be used. The form known as *Datura tatula* has purple stems and flowers while *D. stramonium* has green stems and white flowers. Examine P_1 and F_1 plants, the F_2 population and the sesquihybrid population resulting from a back cross of F_1 on *D. stramonium*. Note distribution into classes in the F_2 and sesquihybrid populations, calculate observed ratios and test goodness of fit as in Exercise 11*a*. Reference: Blakeslee, A. F., in *Jour. Hered.*, viii, 1917.

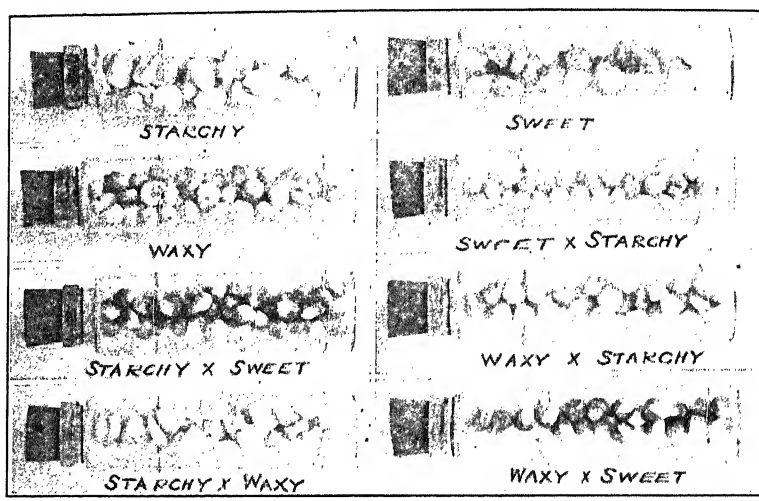


FIG. 2.—Three types of maize grains and the hybrids between them in vials attached to cardboard for class instruction.

12a. Mendelism in Maize—the Grain. II.

1. The contrasted characters dealt with in this exercise are those of endosperm texture or composition. Three types are represented: the starchy endosperm of dent, flint, flour and pop corns; the translucent, wrinkled, sugary endosperm of sweet corns; and the peculiar dull, waxy endosperm of the China waxy corn.

2. Typical examples of these three endosperm types together with the F_1 hybrids produced by cross pollination are furnished in the eight vials fastened to the cardboard (see Fig. 2). Carefully examine first the three endosperm types. Next examine the F_1 hybrid material. What are the immediate results of the following crosses: sweet × starchy, starchy × sweet, waxy × starchy, waxy × sweet? From these results

TABLE IV.—THE F_2 SEGREGATION FOR YELLOW VS. WHITE AND STARCHY VS. WAXY FROM THE CROSS YELLOW STARCHY CORN × WHITE WAXY CORN.

Ear No.	Yellow	White	Starchy	Waxy
1	232	42	217	57
2	199	57	182	74
3	299	59	262	96
4	336	88	331	93
5	347	97	341	103
6	329	95	317	107
7	307	80	289	98
8	337	81	321	97
9	384	102	401	85
10	297	55	276	76
11	274	66	267	73
12	344	79	324	99
13	275	67	256	86
14	336	87	316	107
15	324	77	307	94
16	245	86	253	78
17	349	121	350	120
18	184	69	193	60
19	303	55	260	98
20	268	93	277	84
21	284	68	278	74
22	211	62	201	72
23	240	60	229	71
24	150	37	133	54
25	249	65	224	90
26	311	79	309	81
27	267	79	277	69
28	262	56	231	87
29	242	81	249	74
30	278	76	272	82
31	265	63	248	80
32	147	30	133	44
33	249	74	248	75
34	310	86	321	75
35	270	85	260	95
36	224	57	218	63
37	229	51	214	66
38	261	60	247	74
39	377	92	356	113
40	327	85	340	72
41	240	64	239	65
42	264	77	269	72
43	244	69	262	51

TABLE V.—THE F_2 SEGREGATION FROM THE CROSS YELLOW STARCHY CORN × WHITE WAXY CORN.

Ear No.	Yellow starchy	Yellow waxy	White starchy	White waxy
1	188	44	29	13
2	145	54	37	20
3	214	85	48	11
4	260	76	71	17
5	262	85	79	18
6	247	82	70	25
7	228	79	61	19
8	263	74	58	23
9	312	72	89	13
10	235	62	41	14
11	214	60	53	13
12	265	79	59	20
13	202	73	54	13
14	247	89	69	18
15	248	76	59	18
16	184	61	69	17
17	263	86	87	34
18	143	41	50	19
19	217	86	43	12
20	205	63	72	21
21	225	59	53	15
22	160	51	41	21
23	186	54	43	17
24	110	40	23	14
25	179	70	45	20
26	250	61	59	20
27	216	51	61	18
28	192	70	39	17
29	183	59	66	15
30	213	65	59	17
31	202	63	46	17
32	112	35	21	9
33	195	54	53	21
34	254	56	67	19
35	192	78	68	17
36	180	44	38	19
37	174	55	40	11
38	202	59	45	15
39	288	89	68	24
40	272	55	68	17
41	186	54	53	11
42	210	54	59	18
43	204	40	58	11

what can you say as to dominance in maize hybrids? Which of the crosses show xenia effects?

3. Those envelopes containing grains that exhibit segregation into starchy and waxy are F_2 populations of crosses of either white starchy × white waxy or yellow starchy × yellow waxy. Count three ears, observing same precautions as for the former populations. The segregation of these two endosperm types is distinct, but not so conspicuous as that of starchy and sugary. Accordingly go over each ear two or three times in order to make the correct segregation. Tabulate and make calculations the same as for starchy and sugary endosperm segregation. (NOTE.—If this material is not available the students will deduce the relations as to dominance and recessiveness between starchy and sweet in the first case and between starchy and waxy in the second case by consulting the data given in Tables IV and V.)

4. Give a Mendelian interpretation of your results in a checkerboard representing the factor for waxy by *w* and its allelomorph *W* for starchy. Give in the checkerboard the F_3 segregation ratio for each F_2 genotype.

5. What F_2 segregation ratio was obtained in each of the crosses, starchy × sweet and starchy × waxy? Which character showed dominance? What sort of segregation would you expect in F_2 from the cross waxy × sweet? Illustrate with checkerboard which will show phenotypes.

6. Additional Mendelian work for this exercise deals with the independent dihybrid ratio. Count grains of three ears of assigned material. This material represents the F_2 of a cross between yellow popcorn and a white waxy corn. The F_1 was yellow starchy. Segregate the F_2 grains into four classes: viz., yellow starchy, yellow waxy, white starchy and white waxy. Subject the data thus obtained to the same analysis as that for Exercise 11*a*, except that you test goodness of fit of the observed ratios by using Harris' formula (stated and explained on p. 102 of the text-book) and Elderton's Table (see p. 50). (NOTE.—If it is impossible to furnish the class with hybrid maize grains involving the dihybrid mentioned above, or some other, the data given in Table V may be used. Each student should study the counts of three or four ears as well as the totals of all the ears and criticize the ratios as above directed.)

12b. Mendelism in Tomatoes—the Fruit.

1. Mendelian inheritance exhibited in fruits will be illustrated by preserved specimens of tomato types and their hybrids. The parents used in these hybrids were as follows:

Dwarf Giant—large, round, red
Dwarf Champion—large, round, red
Yellow Cherry—small, round, yellow
Yellow Pear—small, pear-shaped, yellow.

2. In F_2 the populations consisted of 50 plants, each population

derived from a single selfed F_1 fruit. The field counts gave the following data:

Population	Parents	F_2 segregation
15.38	Dwarf Giant × Yellow Cherry	34 red : 14 yellow
15.39	Dwarf Giant × Yellow Cherry	36 red : 12 yellow
15.40	Yellow Cherry × Dwarf Giant	30 red : 18 yellow
15.41	Yellow Cherry × Dwarf Giant	34 red : 14 yellow
15.42	Dwarf Champion × Yellow Cherry	40 red : 10 yellow
15.43	Dwarf Champion × Yellow Cherry	33 red : 15 yellow
15.44	Yellow Cherry × Dwarf Champion	33 red : 15 yellow
15.46	Dwarf Champion × Yellow Pear	24 round red : 7 pear red : 12 round yellow : 6 pear yellow
15.47	Dwarf Champion × Yellow Pear	28 round red : 9 pear red : 8 round yellow ; 3 pear yellow
15.49	Yellow Pear × Dwarf Champion	29 round red : 9 pear red : 7 round yellow : 3 pear yellow
15.50	Yellow Cherry × Yellow Pear	34 round : 12 pear
15.51	Yellow Cherry × Yellow Pear	35 round : 11 pear

3. The jars contain one fruit from each of these plants. Study these preserved specimens critically, especially for the following points: sharpness of segregation as regards red and yellow, round and pear-shaped. Note also segregation with respect to size in these populations. Make full notes, with suggested explanations wherever possible. Discuss the validity of the segregation ratios obtained from the observed frequencies.

4. Make outline drawings of the fruits of P_1, F_1, and F_2 in each of the following crosses. Allow a full page for each cross, and, if possible, color the drawings so that they will give a chart representation of the behavior involved.

(*a*) Yellow Cherry × Yellow Pear

(*b*) Dwarf Giant × Yellow Cherry

(*c*) Dwarf Giant × Yellow Pear

12c. Dihybrid Case in Datura.

By using forms with smooth and spiny capsules as well as purple and green stems the typical dihybrid ratio will be obtained in F_2. These plants can be grown to maturity in 3-inch pots (see Fig. 3).

13a. Mendelism in Maize—the Grain. III.

1. Mendelian studies in maize will be continued with material derived from the cross of two homozygous strains, viz. amber starchy × white sweet. This cross gave amber starchy grains in F_1. Four selfed ears from F_1 gave the following F_2 segregation:

Ear No.	Amber starchy	Yellow starchy	White starchy	Amber and yellow sweet	White sweet
1	268	67	19	86	7
2	250	39	23	92	12
3	186	37	10	84	4
4	214	27	8	59	5
Totals	918	170	60	312	28

2. The Mendelian interpretation of the results depends upon the action of three pairs of allelomorphs; amber *vs.* white, *A* and *a*; yellow *vs.* white, *Y* and *y*; and starchy *vs.* sweet, *S* and *s*. On the basis of such an analysis what would be the formula of the white sweet parent? Of the amber starchy parent? Of the F_1? What segregation ratio would

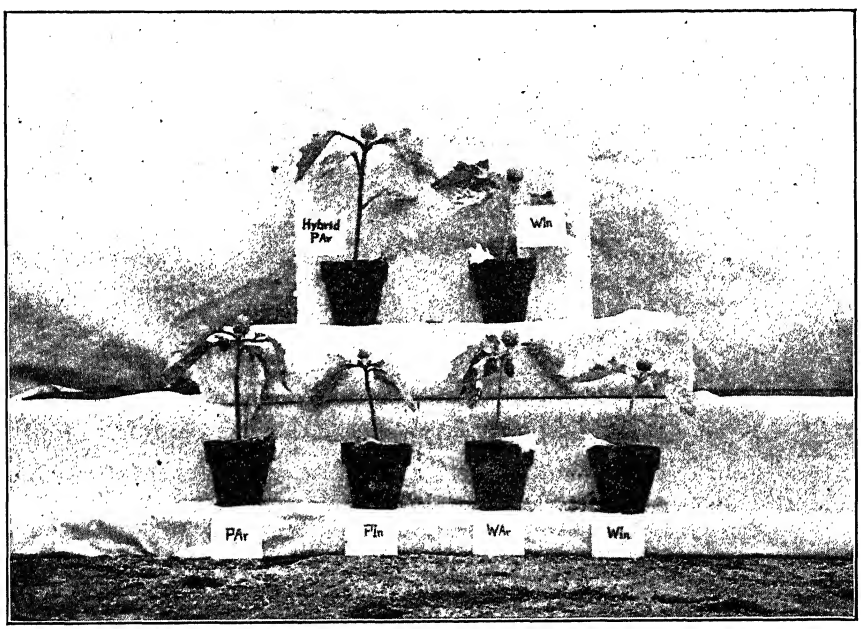


FIG. 3.—Plants of Datura in 3-inch pots showing result of crossing a heterozygous purple armed Jimson weed (Hybrid PAr) with a double recessive or white *inermis* (WIn). The four possible combinations result in a 1:1:1:1 ratio. (*From the Journal of Heredity.*)

you expect to get in F_2? How does this compare with the actual results obtained as illustrated by the four ears?

3. Construct a checkerboard for the F_2 generation which will indicate in each square the phenotypic character of each genotype and also the F_3 segregation ratio which it will produce if selfed.

4. Seeds of the above F_2 classes were planted and the plants selfed

for the F_3 generation, which is represented in the material given you. Segregate the grains of five ears into their ultimate classes with regard to endosperm color; amber, yellow or white; and texture, starchy or sweet. In case of indistinct segregation, as for instance the segregation among sweet kernels into amber and yellow, group the two classes together. Compare the results obtained with the expected results.

5. For each ear that you examine suggest the genotypic formula of the parent, and compare the actual results with those expected from such a genotype both by use of the probable error formula and by Harris' method of testing goodness of fit. In your explanation of the results, state clearly the relation of the factors involved as regards independence in segregation and behavior in phenotypic expression.

(NOTE.—If it is impossible to furnish the class with hybrid maize involving the trihybrid mentioned above, or some other, the data given in Table VI may be used. Study the counts of at least five ears according to directions in the foregoing paragraph.)

13b and c. Mendelism in Other Plants. III.

Excellent material for the study of the trihybrid may be obtained by crossing different types of barley or wheat and such material affords desirable correlation with Exercise 17. The following varieties will give satisfactory results:

Barley.—Nepal × Chevalier. The Nepal is six-rowed, hooded (with abortive awns) and hulless. The Chevalier is two-rowed, awned and hulled.

Barley.—Nepal × Tennessee Winter. This cross will furnish material for study of segregation and recombination in a more complicated series of characters. Besides the characters mentioned above the Nepal is a spring barley and it has the ordinary type of cylindrical spike. The Tennessee Winter is a winter or late barley and the spikes are clubbed.

Barley.—Other varieties which are easily crossed with the above are Beldi and Common California both of which are six-rowed, hulled and awned; Arlington which is strictly awnless; and Guy Mayle which is a black, six-rowed, spring barley. Material of any of the above varieties can probably be obtained by applying to the Bureau of Plant Industry, U. S. Department of Agriculture or from the nearest state agricultural experiment station. The College of Agriculture of the University of California will supply heads as well as grains of these varieties as long as they are available. It is important to secure material that is known to be true to type. Barley is somewhat easier to hybridize than wheat and the characters are very definite and satisfactory for purposes of instruction.

Wheat.—Little Club (or any club variety) × Marquis. The club varieties are spring wheats, having a condensed spike, soft grain, erect culms and awnless. The Marquis is a spring wheat with loose cylindrical spike, hard, red grain, more spreading culms and awnless.

TABLE VI.—F_3 SEGREGATION FROM SELFING F_2 CORN GRAINS FROM THE CROSS AMBER STARCHY × WHITE SWEET

Ear No.	Amber starchy	Yellow starchy	White starchy	Yellow and amber sweet	White sweet
1	118	35	7	66	3
2	...	...	152	...	51
3	208	61	20	89	7
4	203	167	...	111	
5	...	137	...	43	
6	...	38	10	...	11
7	...	572			
8	...	305	88		
9	105	37	7	47	4
10	111	31	...	44	1
11	...	246	93		
12	...	...	...	349	122
13	...	290	...	107	
14	187	56	...	71	
15	...	107	...	34	
16	165	50	6	65	
17	316	124	...	122	
18	259	...	...	141	
19	286				
20	...	235	103		
21	287	81			
22	...	196	67	65	22
23	140	35	13	57	7
24	49	...	9	12	
25	50	25	17		
26	70	...	25	26	
27	208				
28	118	...	...	42	
29	...	182	...	46	
30	...	74	18	23	8
31	...	156	73	59	15
32	202	46	15	86	20
33	...	...	...	...	264
34	...	158	...	49	
35	...	132	...	51	
36	...	325	126	122	
37	...	58	14		
38	...	198	...	54	
39	...	137	75	55	16
40	...	392	81	126	52
41	145	29	...	54	
42	...	...	290	...	107
43	255	71	33	113	6
44	...	...	141	...	57

TABLE VI—*Continued*

Ear No.	Amber starchy	Yellow starchy	White starchy	Yellow and amber sweet	White sweet
45	...	196	66		
46	....	47	24	11	4
47	...	87	27		
48	206				
49	...	323	114		
50	...	...	...	331	98
51	...	...	...	116	
52	...	...	115	...	36
53	263	73	21		
54	...	...	43	...	24
55	37	11	...	25	
56	250	90			
57	...	80	31		
58	...	105	...	31	
59	494				
60	...	...	49	...	18
61	...	...	...	187	
62	75	...	14	23	
63	...	68	32	28	9
64	187	68	22	106	3
65	...	272			

Wheat.—Early Baart or Propo × Marquis. The Early Baart and Propo are spring wheats with soft, reddish grain and awned.

Wheat.—Turkey Red Winter may be crossed with any of the above but not so readily. It is a hard, red, awned winter wheat. The kernel characters differ in all these varieties and will furnish additional data for Mendelian studies. Avoid the use of Sonora wheat in hybridizing to produce material for elementary class use. Some of its characters behave differently from similar characters of the other varieties. It is excellent for more advanced study.

14a, b, c. Species Hybrids.

Material from the tobacco crosses suggested in Exercise 16*b* will be most satisfactory for purposes of illustrating the principles set fortsh in Chapter XII of the text-book, but many other species crosses (some occurring naturally in the wild) will furnish interesting material.

Differences between parent forms, constancy or inconstancy in F_1, characters of progeny from reciprocal crosses, and study of F_2 and sesquihybrid material are the more important considerations.

Make descriptions and drawings of parent and F_1 types and careful notes with sketches of F_2 or sesquihybrid material.

Consider all this with reference to the interpretation suggested in the text-book.

IV. PLANT AND ANIMAL BREEDING

15a, b, c. Hybridization of Plants. I.

I. General Method

The general process includes emasculation and bagging of female parent before flower buds open, protection of pollen parent in the same way, application of ripe pollen to receptive stigma, bagging pollinated flower and attaching labels containing necessary data. The details of hybridization methods will have to be worked out for each species or even for each variety in some cases (see text-book p. 343).

II. Types of Flowers

1. Unisexual flowers from a monœcious plant—Begonia.

(*a*) Describe briefly the cluster, noting method of branching, number of flowers, kinds of flowers, which flowers open first.

(*b*) Draw male and female flowers in longitudinal section, naming the essential organs.

(*c*) Prepare flowers for hybridization. Obviously all that is necessary here is the removal of the male flowers and any open female flowers from a cluster and the covering of the unopened female flowers with a manilla bag. A few days later when the flowers open and the stigma becomes receptive, the bag is removed and pollen from protected flowers of the desired parent plant is applied and the bag replaced until fertilization has been effected. Label, and take notes on the characteristics of the parents.

(*d*) To what economic plants is this simple method of hybridization applicable?

2. Type of an irregular sympetalous flower which is highly specialized for insect pollination—*Schizanthus pinnatus*, or a similar entomophilous species.

(*a*) Lift the entire corolla from the flower noting the position of pistil with reference to lower lip and with scalpel bisect the corolla through the middle line of upper and lower lips. Also bisect pistil and calyx in same plane. Make outline drawings of all parts *in situ* naming same, including ovules.

(*b*) How many pairs of stamens did you observe? How has each pair been specialized? By examining several mature flowers do you conclude that the stamens move? Which? How much? Why? Emasculation of buds consists in removing the entire corolla with stamens and bagging and labeling.

3. Rosaceous Flowers.—As a type study flowers of the strawberry. Study both perfect and imperfect flowers and make drawings to show the essential difference between them. How would you prepare imperfect flowering sorts for cross pollination? Perfect flowering sorts?

III. Hybridization

1. *Leguminous Flowers.*—(*a*) Study flowers of some leguminous plant with special reference to the methods applicable in hybridizing plants possessing this type of flower. The flower of the garden pea is typical for those of the most important subfamily (Papilioneæ) of the Leguminosæ. It includes the clover, peas, beans, soybeans, cowpeas and vetches. Make a large drawing (at least 3 inches long) of a longitudinal section of a flower giving particular attention to the position and arrangement of the essential organs. Label all parts. Count the stamens. How are they arranged in the flower? Make flower plan diagram (cross-section). Determine how best to remove the anthers from an unopened bud and how to use the anthers from a mature flower in pollinating the stigma of an emasculated flower. Do plants with such flowers easily cross-fertilize under natural conditions?

(*b*) *Hybridizing Garden Peas.*—The directions here given apply particularly to the garden pea, but they require no essential modifications for application to other leguminous plants such as sweet peas, beans, etc.

Emasculation.—For crossing purposes select buds in which the anthers have not yet opened. Carefully slit the keel lengthwise along one side with the forceps, thus exposing the anthers. Remove the anthers with the forceps, taking care not to injure the stigma. In order to be sure that all the anthers are removed, it is well to count them as they are taken out. The corolla should ordinarily be injured as little as possible in this operation, although in most species such injury is not a serious matter.

Bagging.—After the anthers have been removed, cover the flowers with a paper bag, tying it with a piece of string. Label the plant with your name and the date of emasculation.

Pollination.—Remove bags one at a time and if the stigma is receptive, pollinate with the desired pollen of known purity and immediately cover again and leave the bag on until fertilization has been accomplished as indicated by the withered or brownish stigma. In order to be certain of the purity of the pollen, flowers from which it is taken should have been bagged in the bud stage at the same time that the buds of the female parent were emasculated. It is best to remove bags when the fruit has "set." As far as possible reciprocal crosses should be made. In careful work it is necessary to wash the hands and instruments in strong alcohol after each operation.

Recording.—The notebook record should include a description of each parent form, giving particular attention to the contrasted characters. The female parent should always be written first. The record on the label should include variety name or number of each parent, date of emasculation and pollination, and name of student.

16a. Hybridization of Plants. IIa.

1. *Datura,* Jimson (Jamestown) weed. In preparation for hybridization study the flower structure carefully. The material secured from the crosses to be made may be used in later Mendelian studies. Note relative position of essential organs during anthesis with reference to natural self- or cross-fertilization. Fully describe the mature flower, making a large drawing of a longitudinal section, labeling all parts; also a diagram of floral plan, and a much enlarged diagram of longitudinal and cross-sections of the ovary as seen under a low-power lens. Examine a flower bud which is in about the proper stage for emasculation.

2. In the greenhouse or laboratory prepare the bud or buds on one plant of each of the varieties that are to be crossed (see Exercises 11*b* and 12*c*). With a sharp-pointed forceps slit the corolla for about half its length thus exposing the immature anthers. Pull out the anthers counting them as they are removed and using care not to injure the pistil. Cover the emasculated bud with a bag fastened with a wire label bearing necessary data.

3. Pollinate 2 or 3 days later when the corolla of the emasculated flower has expanded. This may be done by seizing an anther of the desired male parent with the forceps and applying the pollen directly to the stigma. The flowers from which pollen is taken should be protected in critical work. After pollination write on the label the numbers of female and male parents, date, your name and number of operation if more than one is performed.

16b. Hybridization of Plants. IIb.

1. *Nicotiana Species.*—In preparation for hybridization study the tobacco flower. Suggested material: *Nicotiana langsdorffii* var. *grandiflora.* Although this form is not used commercially, its flowers do not differ essentially from those of the common commercial tobaccos except that they are large in size. If this form is not available use flowers of *N. macrophylla.* Study and describe the mature flowers, noting relative position of essential organs in anthesis with reference to occurrence of self- or cross-fertilization. Make a large drawing of a longitudinal section, labeling all parts; also diagram of floral plan and a much enlarged diagram of longitudinal and cross-sections of the ovary as seen under a low-power lens. Examine a flower bud with reference to emasculation.

2. In the greenhouse or laboratory prepare one shoot of the variety furnished for the hybridization work. Suggested material: *Nicotiana macrophylla,* one plant for each student; pollen parents, *N. tabacum, N. sylvestris,* or others.

Snip off all open flowers and young seed capsules and all the flower buds except one or two which are saved for the work. Rub off the small buds on the side of the shoot so as to prevent the growth of young

branches later on. The buds which have been saved should be just ready to expand, the anthers should be full sized but should not yet have dehisced. With a sharp-pointed forceps slit the corolla for about half its length, thus exposing the immature anthers. Then pull out the anthers, counting them as they are removed. Do not injure the pistil during the operation. After emasculation, enclose the buds in a paper bag securely tied around the stem with a wire label.

After completing the work of emasculation take careful notes on the differences between the intended female and male parents.

3. Pollinate 3 or 4 days later when the corolla of the emasculated flower has expanded. This is done by seizing a ripe anther of the desired male parent with the forceps and dusting the pollen directly onto the stigma. Enclose the flowers which are to be used for the pollen parent in a bag before they open in order to protect them from contamination with foreign pollen. After pollination write the necessary legend on the label, including number of female and male parent, date, your name and number of operation.

16c. Hybridization of Plants. IIc. Tomato or Potato.

Study flowers and make crosses according to directions in 16*a* and *b*. When examining the flower note especially the arrangement of the essential organs with reference to natural self-pollination. How do the anthers discharge their pollen? Would you expect to find pure lines in tomatoes? In potatoes? Why or why not?

17a, b, c. Hybridization of Plants. III.

Flowers of the grasses: wheat, barley, oats, etc.

Although the following directions apply particularly to wheat, with slight modifications they can be adapted for other members of this family.

1. A spike of wheat consists of a compressed stem or rachis bearing the spikelets alternately. Each spikelet has from three to five flowers. The flower consists of (*a*) the protective parts; the outer one is called the flowering glume and bears the awn or beard while the inner one is the palea or palet, a thin scale with two nerves; (*b*) the essential parts, the stamens and pistil. Note that there are three stamens, each consisting of a filament bearing a four-chambered anther at its summit. The pistil consists of an ovary with a single ovule, and two feathery plumose stigmas. Sketch a flower much enlarged to illustrate the relation of the essential organs to each other.

2. The following directions apply specifically for hybridization in wheat. It is necessary to modify them somewhat in working with barley, oats, or rice. Strip off the basal spikelets amounting to about one-third of the head and also clip off the top third of the head. The four or five remaining spikelets contain three to five flowers each. Of these all except the outer pair of each spikelet are removed, thus reducing the ear

to 12 to 20 flowers. Now gently insert the point of the closed forceps between the upper margins of the palet and flowering glume. By releasing the pressure on the forceps the 3 anthers and the feathery stigma are exposed to view. Remove these anthers. With practice this may be done with a single stroke of the forceps. If the anthers are shedding pollen the flower must of course be rejected and the forceps sterilized before operating on the next flower. The best stage at which to carry out the operation is when the anthers are just approaching maturity but are still green; when yellow they are too likely to burst. Having removed all the anthers, wrap the head in cotton cloth or cover it with a paper bag until the stigmas become receptive. Then remove the covering, and, from the desired male parent, secure ripe anthers just ready to burst. Grasp these with a fine pair of forceps, break them in halves, and dust the contents over the feathery stigma of each flower in succession. In case the pollen is not in good condition some of the anthers may be broken and left inside the protective glumes of the flowers. The stigmas remain receptive for several days. Although a single pollen grain is sufficient to effect fertilization, it is certain that the presence of a liberal quantity of pollen on the stigma stimulates development.

3. Emasculation and pollination may be done at one operation. It is necessary in such cases to choose fairly mature flowers, and greater care must be taken to avoid self-fertilization. Any flowers in which the anthers have already liberated pollen should be removed.

4. After pollination replace the protective covering to prevent the entry of foreign pollen. Then label the head with the names of the female and male parents, date of cross, etc., and support it with a stake if necessary. It is well to remove this protective covering about 2 weeks after pollination so that it will not interfere with normal ripening.

5. In all hybridization work with small grains it is important to make repeated check experiments to assure yourself that self-pollination does not occur, or if it does, to what extent. Any apparently self-fertilized grains secured should also be tested. Sometimes it will be found that they are not viable and hence probably resulted from parthenocarpy.

18a, b, c. Live Stock Registration.

I. Original Registration of Pure Bred Animals

1. Many years of careful breeding and selection for an ideal type has produced many excellent lines or families of live stock which are known to transmit most of their desired qualities to their progeny.

2. The first man to apply scientific methods to cattle breeding was an Englishman, Robert Bakewell. The facts gleaned from his experiments were used in the improvement of the Shorthorn breed by the Colling brothers who began their work in England about 1780. The superiority of their cattle demonstrated the value of knowing the ancestors and performance records when matings were made, and consequently

APPLICATION FORM FOR REGISTRY IN AMERICAN SHORTHORN HERD BOOK

Names containing more than eighteen letters including name numbers will not be accepted for record—the shorter the better.

SEX Male PRIVATE NUMBER

Name Legal Choice (Give name of animal to be recorded on this line) Color red calved Nov. 11, 1914

bred by J. E. Barr (The Breeder of an animal is the Owner of dam at time of service.) P. O. Davenport State Iowa

owned by J. E. Barr P. O. Davenport State Iowa

Sire (Give name and number of sire on this line.) No. 304699

Dam New Year's Pride, H.B. 63,P. 562 (Give name and number of dam on this line.) No.

Dam's Sire Golden Crown (Give name and number of sire of dam on this line.) No. 127851

If this pedigree traces to an imported dam, write her name below for the Secretary's convenience in verifying.

Tracing to Imp. Dam

Imp. Dam's Sire

When signing this application the person doing so subscribes to the truth of it to the best of his knowledge and belief. Dated 1915

In case the dam was purchased carrying calf for which this application is made, the party owning dam at time the calf was dropped must sign this application here in substantiation of color and birthdate.

This application must be signed here by the breeder.

Signature of Breeder.

If the owner of the sire of the calf for which this application is made was not also the owner of the dam at time of service, then his signature is required here in addition to breeder's signature opposite.

Or in case of death of breeder, application must be signed here by proper representative, either administrator, heir or herd manager.

AMERICAN SHORTHORN BREEDERS' ASSOCIATION, 13 DEXTER PARK AVE., CHICAGO

8884

FIG. 4.—An application form for original registration.

more attention was given to the recording of matings made with any of these improved or pedigreed families. The first herdbook for

A cross mark placed in front of any of the numbers below will indicate the reason this application is returned.

Refer to File No._____

1. This animal already recorded under the number _____
2. Make application on attached form. See instructions enclosed.
3. Name longer than allowed. (Not more than 4 words or 18 letters allowed.)
4. Animal of same name by this sire already recorded. Please give another name. No name given. Please furnish.
5. Animal's name number should be higher than the name number of the dam or sire. Name numbers or letters out of order. Example: A cow born Jan. 1, 1915, might be named Mary. Mary 2d should be calved since that date, and Mary 3d after Mary 2d. Same applies where letters A, B, C, etc., are used instead of 2d, 3d, 4th, etc. We never print the name number "1st."
6. This pedigree not recorded on account of death of animal.
7. This pedigree returned with the pedigree of the sire or dam.
8. If a twin, please so state on application.
9. Registration not made within time limit. See rule 7. Please remit _____
10. Sex not indicated or does not agree with name.
11. Color description omitted.
12. Birthplace incorrect—Incomplete—Omitted.
13. Sire's name and number omitted or incorrect.
14. Dam's name and number omitted or incorrect.
15. Give name of sire of dam.
16. The Breeder is the owner of the dam at time of service.

    According to our records the dam was owned by

    at time of service. If you are the breeder of the

    calf, please obtain a signed transfer application from the seller, unless you owned the dam prior to March 1, 1915. See Rule 14.
17. According to our records you were not the owner of the sire at time of

    service. Sire according to our records, owned by

    at time of service. Please obtain a transfer if you

    own the sire or obtain signature of the owner on application for calf. See Rule 14 and Page 7.
18. We have an animal on record named

    born bred by

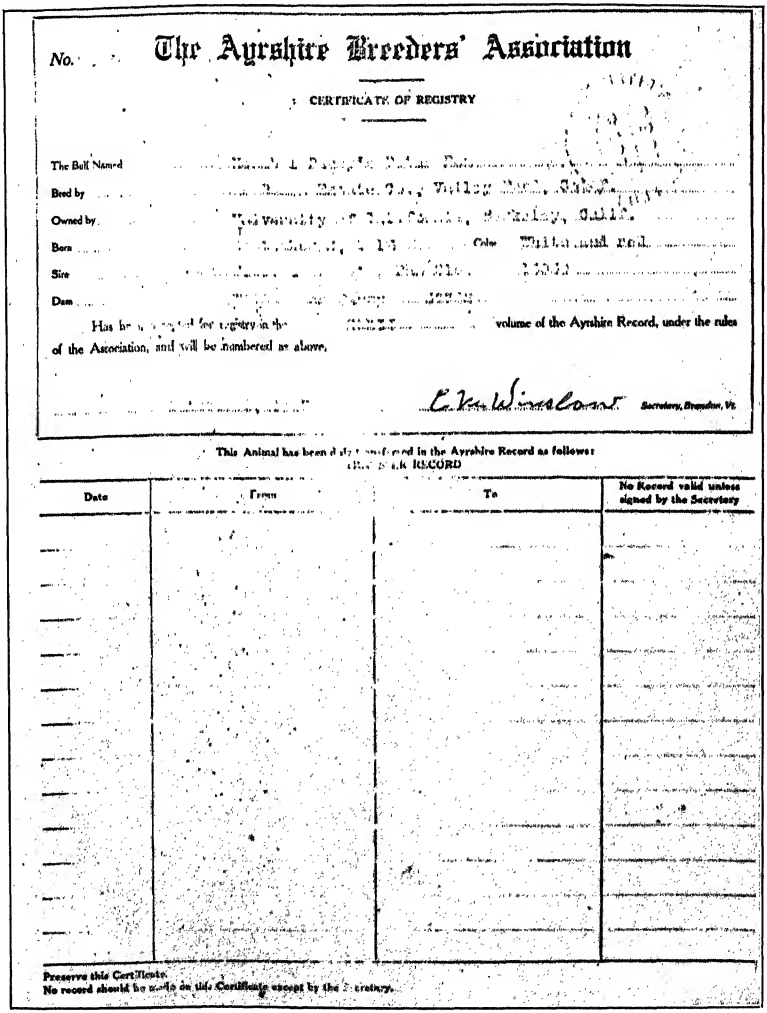
    therefore cannot record this pedigree without satisfactory explanation.
19. Our records show was less than 15 months of age when this calf was born. Please explain.
20. Breeder's signature must be obtained. See Number 16 above.
21. Owner of dam at time of birth of animal must sign application.
22. The signature does not appear to be that of _____
23. When signing for firm or for anyone else, sign your own name underneath and state in what capacity you are signing.
24. Please furnish correct post office address of the owner, breeder,
25. Please make declaration for duplicate. Blank form attached. Fee 25 cents. or 50 cents if transfer is required. See Rule 13.
26. Fees were not sufficient. Shortage _____

AMERICAN SHORTHORN BREEDERS' ASSOCIATION
13 Dexter Park Avenue, Chicago

FIG. 5.—Reverse of application blank shown in Fig. 4. Note requirements for registration.

recording Shorthorn cattle was established in England in 1822, and the first one in America in 1846. The latter now includes nearly 100

volumes. In the preface of each are the rules governing the entry of animals. The Breeder's Associations of the various kinds of live stock now each publish a herdbook (flock book for sheep) containing records of the pedigreed animals of that breed.



No. The Ayrshire Breeders' Association

CERTIFICATE OF REGISTRY

The Bull Named

Bred by

Owned by

Born Color

Sire

Dam

Has been registered for registry in the volume of the Ayrshire Record, under the rules of the Association, and will be numbered as above.

Secretary, Brandon, Vt.

This Animal has been duly transferred in the Ayrshire Record as follows:

TRANSFER RECORD

Date	From	To	No Record valid unless signed by the Secretary

Preserve this Certificate.
No record should be made on this Certificate except by the Secretary.

FIG. 6.—Certificate of registry of an Ayrshire bull.

3. Registration consists in making application for registry and the issuing of a properly signed certificate of registry. The application is made by the owner or breeder on blanks furnished by the Association (see Figs. 4 and 5). A diagram or description of the animals showing exact location and amount of colored areas must be furnished with the

application for registry. Only animals from registered parents may be registered. The certificate of registry bears a reproduction of the diagram or description of color markings furnished by the breeder. When a recorded animal is sold the certificate of registry is sent to the Association secretary and the transfer recorded in the Association records

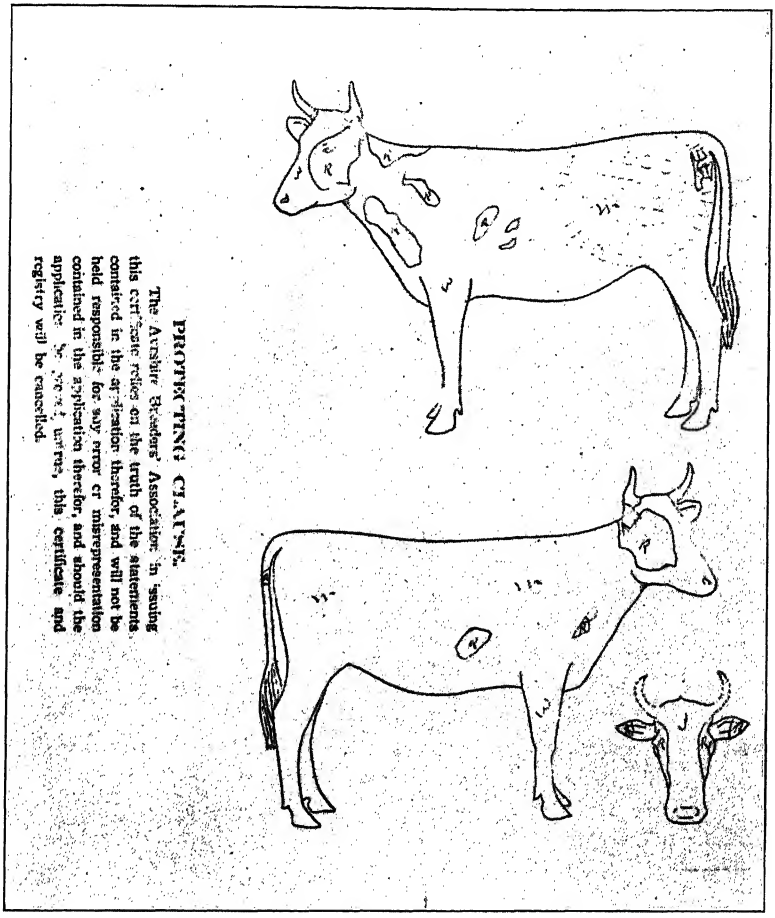


FIG. 7.—Reverse of certificate shown in Fig. 6. Diagram shows color markings of the Ayrshire bull, Norabel Pansy's Peter Pan 20385.

and on the certificate of registry itself. Most certificates have a printed form attached for this purpose (see Fig. 6). Fig. 6 is a facsimile of the certificate of registry of the Ayrshire bull, Norabel Pansy's Peter Pan, 20385, owned by the University of California. No record of transfer is shown as his original registration was made after he became the property of the University.

4. The superiority or excellence of an animal depends not upon the fact that a certificate of registry can be shown but upon immediate ancestral connection with animals of marked merit.

FORM K NO. 518—9M-6-16-16-R

HOLSTEIN-FRIESIAN ADVANCED REGISTRY

Owner's Application for Permission to Officially test Cows for Entry in the Advanced Register

(Do not omit postoffice address)

Mr. M. H. Gardner, Supt. A. R.,
Delavan, Wis.

.....191....

Dear Sir:

In accordance with our rules, I hereby make application to you, as Superintendent of Advanced Registry, for permission to officially test certain cows hereinafter listed by name and number, should all go well with them. It is my intention to apply for the admission of these cows to advanced registration, or for the admission of such part of them as may qualify under our rules. I expect to apply to.....the officer in charge of tests of dairy cows at .....Agricultural College for a supervisor to arrive about ..... to conduct the tests of the bunch herein listed.

NAME	NUMBER	AGE	PROBABLE DATE OF FRESHENING

I know that by the action of the Board of Directors of the Holstein-Friesian Association of America, the owner, or person in charge, is now held responsible for the making of partial preliminary reports, either in person or through the supervisor in charge of the conduct of the test, to the Superintendent of Advanced Registry during the progress of all tests showing productions above certain minimum amounts for the age, that the closing of any test on which a verification test might be ordered is not to be held as any bar to such verification test; and that the responsibility for so feeding and caring for any cow under test as to keep her fit for re-test is placed wholly upon the owner. I make this application subject to all existing rules and regulations adopted by the Holstein-Friesian Association of America relating to the making of official and semi-official tests and the acceptance and entry thereof in the Advanced Register, and also subject to all existing By-Laws of the Association.

(Signed) .....
Owner.

(Signed) .....
Person in Charge.

Note—If owner signs report, he is to sign in space for owner. If person in charge signs, he is to use the proper space, but is also to give the name of the owner. Enter the name of each cow in full, and be sure to get the number correct.

FIG. 8.—An application blank for advanced registry.

5. Using blank application forms make out complete application for the registration of an assigned animal; the name and herdbook

volume being given. Locate the animal in the index of the given volume, then trace out the necessary individuals and data needed for the application. Where possible color markings should be indicated by diagram.

(NOTE.—Application blanks may be secured from live stock breeders' associations or may be mimeographed for class use. Following is a list of the Secretaries of the dairy breed associations.)

Ayrshire breed.....Secretary, C. M. Winslow, Brandon, Vt.
Brown Swiss breed.....Secretary, Ira Inman, Beloit, Wis.
Guernsey breed.....Secretary, Wm. H. Caldwell, Peterboro, N. H.
Holstein-Friesian breed..Superintendent, M. H. Gardner, Delavan, Wis.
Jersey breed..Secretary, R. M. Gow, 324 W. Twenty-third St., New York.

The office of the American Shorthorn Breeders' Association is located at Union Stock Yards, Chicago, Ill. For addresses of all other American breeders' associations, see Curtis, "Live Stock Judging and Selection."

II. Advanced Registry

1. *Definition.*—The advanced registration of live stock is based upon individual merit and performance and is designed as an aid to improvement within a breed. It is especially adapted to the improvement of dairy herds. Any pedigreed animal may, on showing the required degree of merit, be given advanced registration. The individual excellence is measured on the part of the cow by her ability in dairy production, and on the part of the bull by his potency in the production of advance registry daughters. Grade cows are sometimes given the tests for advance registry but since the value of their offspring as dairy producers cannot be depended upon, ancestry being obscure, the expense attached to securing advanced registry is seldom warranted. At the present time the Guernsey, Jersey, Holstein, Ayrshire and Brown Swiss breed associations have adopted this plan of advance registration. The requirements for cows vary slightly as shown in Table VII.

TABLE VII.—REQUIREMENTS FOR ADMISSION TO THE ADVANCED REGISTERS OF BREED ASSOCIATIONS (FROM UNIV. CAL. A. E. S. CIR. 135)

Age	Ayrshire year record		Brown Swiss year record		Guernsey	Holstein	Jersey	
	Pounds milk	Pounds butter fat	Pounds milk	Pounds butter fat	Year record, pounds butter fat	7-day record, pounds butter fat	7-day record, pounds butter fat	Year record, pounds butter fat
2 years	6,000	214.3	*6,000	*222.0	*250.5	7.2	12.0	250.5
3 years	6,500	236.0	6,430	238.5	287.0	8.8	12.0	287.0
4 years	7,500	279.0	7,288	271.3	323.5	10.4	12.0	323.5
5 years	8,500	322.0	8,146	304.2	360.0	12.0	12.0	360.0
6 years	.....	.....	9,000	337.0	.....	.....	.....	.....
Pounds increase per day over minimum	†1.37 and ‡2.74	†.06 and ‡.12	2.35	.09	.1	.00439	.....	.1

*Two and one-half years. †For cows in 2-year-old form. ‡For cows in 3-year-old form.

2. *Requirements for Entry of Bulls (Holstein).*—Only bulls having not less than four A. R. O. daughters are eligible to entry in the Advanced Register; and the Superintendent will, without any special application

Form 4. No. 507—10M—7-14-16—R

HOLSTEIN-FRIESIAN ADVANCED REGISTRY

APPLICATION FOR ENTRY ON AN OFFICIAL RECORD

Of the Holstein-Friesian Cow Aralia De Kol - Mead H. B. No. 182592

Owner A. W. Morris & Sons Corp Address Woodland Cal.

Born July 23 191[illegible] Dropped last calf March 31 1917

CERTIFIED YIELD OF MILK AND FAT

DATES	1 A. M. Lbs. Milk	1 A. M. Per Ct. Fat	1 A. M. Lbs. Fat	7 A. M. Lbs. Milk	7 A. M. Per Ct. Fat	7 A. M. Lbs. Fat	1 P. M. Lbs. Milk	1 P. M. Per Ct. Fat	1 P. M. Lbs. Fat	7 P. M. Lbs. Milk	7 P. M. Per Ct. Fat	7 P. M. Lbs. Fat	TOTAL Lbs. Milk	TOTAL Lbs. Fat
April 1	15.7	4.3	.675	17.3	4.0	.692	17.6	3.8	.669	17.6	3.7	.655	68.2	2.687
2	16.9	4.2	.710	16.8	4.25	.714	17.8	4.3	.765	16.8	4.1	.689	68.3	2.878
3	17.3	4.0	.692	15.4	4.1	.754	18.1	3.95	.715	18.0	3.9	.693	71.8	2.854
4	17.6	3.9	.686	19.6	3.75	.735	18.8	3.85	.724	20.1	3.8	.769	76.1	2.909
5	18.3	3.95	.723	19.4	3.8	.739	19.5	3.9	.761	20.7	3.65	.756	77.9	2.977
6	17.7	3.7	.655	19.2	3.85	.739	19.4	3.8	.739	19.7	3.8	.749	76.0	2.880
7	19.2	4.0	.768	18.9	3.8	.718	20.0	3.9	.780	20.4	3.9	.796	78.5	3.062
8	18.0	4.1	.738	19.1	4.05	.774	20.5	3.9	.800	20.7	3.85	.799	78.3	3.109
9	20.0	3.75	.750	19.9	4.05	.806	19.3	4.1	.791	19.9	4.0	.796	79.1	3.143
10	19.7	3.8	.749	19.8	4.15	.822	18.7	4.3	.804	19.1	3.85	.735	77.3	3.110
11	18.4	4.1	.754	18.3	4.25	.778	19.2	4.2	.806	19.7	4.05	.798	75.6	3.136
12	17.3	4.05	.701	18.4	4.1	.754	19.7	4.2	.827	19.6	4.35	.853	75.0	3.135
13	17.8	4.6	.819	17.0	4.4	.748	18.3	4.5	.824	17.4	4.2	.731	70.5	3.122
14	15.3	4.4	.673	16.7	4.4	.735	15.6	4.5	.702	13.4	5.0	.670	61.0	2.780
15	12.5	5.2	.650	14.0	6.5	.910	13.0	5.9	.767	14.3	4.8	.686	53.8	3.013
16	13.6	4.4	.598	14.7	4.4	.647	14.2	4.1	.582	16.0	3.8	.608	58.5	2.435
17	16.0	3.9	.624	18.2	3.8	.692	16.7	4.2	.701	17.6	3.9	.686	68.5	2.703
18	18.0	3.4	.612	19.2	4.35	.835	18.6	4.0	.744	19.8	3.7	.735	75.6	2.924
19	18.6	3.5	.651	20.0	3.6	.720	21.1	3.75	.791	19.4	3.7	.718	79.1	2.880
20	19.2	3.55	.682	18.2	3.65	.664	19.0	3.6	.684	20.4	4.1	.836	76.8	2.866
21	18.1	3.5	.634	20.4	3.65	.745	18.6	3.9	.725	19.5	3.9	.760	76.6	2.865
22	17.2	3.3	.568	20.3	3.55	.721	20.2	4.0	.808	20.8	4.05	.842	78.5	2.939
23	19.8	3.6	.713	18.0	3.4	.612	20.9	3.55	.742	20.1	3.6	.724	78.8	2.791
24	18.4	3.1	.570	19.3	3.7	.714	20.8	3.9	.811	17.2	3.1	.533	75.7	2.628
25	21.9	3.5	.766	16.5	3.8	.627	15.1	3.3	.498	19.7	2.9	.571	73.2	2.463
May 1	15.1	3.0	.453	19.8	4.2	.832	20.9	4.7	.982	20.3	5.7	1.157	76.9	3.324
2	19.7	4.9	.965	20.0	4.4	.880	18.7	4.2	.785	19.2	3.9	.749	77.4	3.379
3	21.1	3.4	.719	21.6	4.0	.864	20.6	3.9	.803	20.3	3.7	.751	83.6	3.135
4	20.1	3.1	.623	21.0	3.6	.756	20.0	3.8	.760	22.4	3.65	.818	83.5	2.957
5	20.3	3.65	.741	21.5	3.85	.828	18.8	3.6	.677	21.5	3.4	.731	82.1	2.977
31														
												TOTAL	2232.4	88.061

This blank to be used for reporting tests in excess of seven days—lengthen if necessary.

FIG. 9.—Data from an official 30-day dairy test.

having been made, make entry of all bulls as soon as they have the required number (4) of A. R. O. daughters. An A. R. O. daughter is one that has been entered in the Advanced Register on an official test.

3. *Advance Registry Tests.*[1]—(*a*) Official tests, usually cover periods from 7 to 30 consecutive days during which the official Supervisor is present at every milking and records the data.

No. 547—10M—7-14-16—R

HOLSTEIN-FRIESIAN ADVANCED REGISTRY

REPORT OF THE

OFFICIAL BUTTER FAT TEST

Of the Holstein-Friesian Cow Oralia Jakol Mead H. B. No. 182592

Owned by A. W. Morris & Sons Co. born July 23 1914

Dropped last calf March 31 1917 Strictly official for year record. Also semi-official (?) yes 1 also

SUMMARIES OF PRODUCTION

Length of Record	Date and Hour From	Date and Hour To	Milk Pounds	Butter Fat Per Ct.	Butter Fat Pounds
7 Days	7Am. April 11	7Am April 18	539.9	4.02	21.739
30 Days	12M April 6	7Pm May 5	2232.4	3.94	88.061
60 Days					
( ) Days					

I have kept all untested samples securely fastened under lock and key. The weights, tests and calculations, as given above and on the reverse side of this report, were made by me, and the results reported are true and correct to the best of my knowledge and belief. The Certificate of Registration of the above cow has been examined by me, the diagrams thereon, or the description therein, substantially agreeing with the markings of the cow; and in the making of this test all the rules of my State Agricultural College have been strictly followed.

Given under my hand this 15th day of May 1917

Joseph P. Hartel Supervisor of Test.

Subscribed and sworn to before me

this day of 191

SWORN STATEMENT

Vouched for by F. W. Woll

of California Agricultural College.

(All rules of the H.-F. Association covering the test of the above cow have been complied with, all information given concerning her is correct, and this application is made subject to all existing Rules and By-Laws of the Association.

Frank L. Morris for

Subscribed and sworn to before me A. W. Morris & Sons Co. Owner of Cow.

this 15th day of May 1917 A. J. Morris Feeder.

C. W. Thomas Notary Public A. J. Morris Milker.

(Seal)

Except when, near the close of the fiscal year, one may desire to make a special report of a test period constituting a portion of a test, in order that it may compete for prize-money, a detailed report should not be made to the Superintendent until a test is closed; but whenever the yield equals or exceeds the minimum requirements for partial preliminary reports, such reports must be made to the Superintendent that he may order a re-test if deemed desirable. The re-test may form a portion of any official test, if there be no break in the test.

Whenever two Supervisors may take part in the conduct of any official test, the one relieving the other, the table of certified yield of milk and fat should be continuous, and a star in red ink mark the change in Supervisors.

Use one decimal place in the entry of milk, and three decimal places in the entry of fat.

FIG. 10.—Reverse of report shown in Fig. 9.

(*b*) Semi-official tests are conducted usually for yearly records, the milk and butter-fat production of the cows for 2 consecutive days in every month is determined as in official tests, the owner submits daily records at the end of each month. In the Holstein-Friesian breed form 6

[1] For complete rules and instructions regarding individual cases apply to the agricultural college or to the secretary of the particular live stock breeders' association. For some addresses see p. 41.

(not illustrated) is used for this purpose. Records of tests should in each case be accompanied by a diagram showing exact color markings of the cow being tested.

Form No. 3 No. 507—1M—7-27-16

HOLSTEIN-FRIESIAN ADVANCED REGISTRY

APPLICATION FOR ENTRY ON AN OFFICIAL RECORD
Made at Least Eight Months After Calving

Of the Holstein-Friesian Cow. Ayalia Tensal Mead H. B. No. 182592
Owner A. W. Morris & Sons Corp. Address Woodland Cal.
Born 7 2-3 1911 Dropped last calf 3 3-4 1917
for the seven days from 7 P.M. Jan 17th 1918 to 1 P.M. Jan 24th 1918

CERTIFIED YIELD OF MILK AND FAT.

DATES	1 A.M. Lbs. Milk	1 A.M. Per Ct. Fat	1 A.M. Lbs. Fat	7 A.M. Lbs. Milk	7 A.M. Per Ct. Fat	7 A.M. Lbs. Fat	1 P.M. Lbs. Milk	1 P.M. Per Ct. Fat	1 P.M. Lbs. Fat	7 P.M. Lbs. Milk	7 P.M. Per Ct. Fat	7 P.M. Lbs. Fat	TOTAL Lbs. Milk	TOTAL Lbs. Fat
Jan 17										15.0	6.4	.960	15.0	.960
18	18.6	4.3	.800	15.8	4.1	.648	14.4	5.0	.720	14.6	5.8	.847	63.4	3.015
19	14.2	4.8	.682	12.8	4.0	.512	15.3	5.5	.842	13.0	5.75	.748	55.3	2.784
20	14.1	4.9	.691	14.0	5.2	.728	13.8	4.6	.635	16.0	5.6	.840	56.9	2.894
21	15.0	5.0	.750	14.2	5.0	.710	14.0	4.5	.630	13.9	5.3	.737	57.1	2.827
22	14.2	4.5	.639	14.0	4.5	.630	12.9	5.0	.645	15.1	5.85	.883	56.2	2.797
23	13.0	4.9	.637	14.6	3.8	.555	15.1	5.4	.815	14.8	6.4	.947	57.5	2.954
24	14.6	4.9	.715	14.5	4.3	.624	17.6	5.05	.889					

Total yield for seven days was 408.1 lbs. milk containing 20.459 lbs. fat.

I have kept all untested samples securely fastened under lock and key. The weights, tests and calculations in the above table were made by me, and the results reported are true and correct to the best of my knowledge and belief. The Certificate of Registration of the above cow has been examined by me, the diagrams thereon, or the description therein, substantially agreeing with the markings of the cow; and in the making of this test all the rules of my State Agricultural College have been strictly followed.

Given under my hand this 31 day of January 1918
R. P. Penfield
Supervisor of Test.

Subscribed and sworn to before me this _____ day of _____ 191_____
SWORN STATEMENT
_____

Vouched for by F. W. Woll
of California Agricultural College.

Has this cow aborted since earlier record of this period of Lactation? No

Has she been bred? Yes.

If so, please give date of service August 2nd, 1917

All rules of the H.-F. Association covering the test of the above cow have been complied with, all information given concerning her is correct, and this application is made subject to all existing Rules and By-Laws.

Subscribed and sworn to before me this 5th day of Feb 1918
C. W. Thomas

A. W. Morris & Sons Corp.
By A. W. Morris Owner of Cow.
A. J. Morris Feeder.
A. J. Morris Milker.

FIG. 11.—Official data showing persistency of production in a dairy cow.

Official tests for advance registry are conducted under the supervision of the State Agricultural College.

4. Using forms such as here illustrated complete for a 7-day period the official records for the advance registration of an animal as-

signed and using data furnished by instructor.[1] Use blank form K for application, forms 4 and 7 (Figs. 9 and 12) for official test records. Form 4 is also used in recording semi-official tests and form 3½ (Fig. 11) is used in recording an official test of presistency in production.

Form 7 No. 535—10M—11-15-17

HOLSTEIN-FRIESIAN ADVANCED REGISTRY

DEPARTMENT OF SEMI-OFFICIAL TESTS

Detailed Report of the Official Test of the Holstein-Friesian Cow

Name Aralia De Kol Mead 2d Number 326061

Owner A. W. Morris & Sons Corp. Address Woodland, Cal

for two days in the Month of March, 1918

as tabulated below:

Date	Milkings*	Milk (In lbs. and tenths)	Per Cent of Fat 1st test	Per Cent of Fat 2nd test	Fat in Milk (In lbs. and thousandths)
Mar. 18	1 am	13.7	3.7	3.7	.507
	7 A.M.	14.7	3.8	3.8	.536
	1 P.m.	13.0	3.8	3.9	.501
	7 P.M.	12.7	3.8	3.8	.483
" 19	1 a.m.	14.5	3.0	3.0	.435
	7 A.M.	12.2	3.8	3.9	.470
	1 P.M.	13.0	3.5	3.5	.455
	7 P.M.	14.6	3.7	3.8	.548

*In case of four milkings, place two of them in one of the spaces, one above the other. Report test in full. Selected period not allowable; see paragraphs 5 and 6 on reverse side of this sheet.

The total yield for the two days was 107.8 lbs. milk, containing 3.935 lbs. fat.

The average per cent fat for the total milk produced was 3.65

WEIGHTS OF FIVE DAYS' MILK PREVIOUS TO TEST.

Month and Day	Lbs. Milk 7 A.M.	7 Noon	1 P.M.	7 P.M.	Total
Mar 13	13.7	11.1	12.6	12.2	59.6
" 14	13.2	12.5	12.6	13.2	51.5
" 15	14.5	12.5	13.2	13.3	54.5
" 16	14.7	13.7	13.2	13.3	54.4
" 17	12.7	12.8	13.4	13.4	52.3
			Total five days' milk		272.3

The weights, tests and calculations in the above table were made by me, and the same are true and correct to the best of my knowledge and belief. The Certificate of Registration of the above cow has been examined by me, the diagrams thereon substantially agreeing with the markings of the cow. I have kept all untested samples securely fastened under lock and key.

Given under my hand this 19th day of March 1918

W. L. Kelsey.
Supervisor of test.

Subscribed and sworn to before me this 21st day of March 1918

C. W. Thomas.

Vouched for by F. W. Woll of California Experiment Station.

FIG. 12.—Official data for one month of a long term semi-official test for advanced registry.

5. From data of paragraph 4 supply the official records for 1 month of a year term semi-official test of a dairy cow, using forms such as

[1] If instructor has no available data use may be made of that given in Fig. 9.

those illustrated in this exercise. Use form 7 (Fig. 12) for monthly report of semi-official tests.

19a, b, c. Live Stock Pedigrees.

1. Pedigrees of pure bred live stock are recorded in herdbooks which are published by breeders' associations. The present exercise is designed to familiarize students with methods of tracing pedigrees and estimating the degree of inbreeding and of relationship which they indicate. The examples for this work have been selected from the Shorthorn breed.

2. Shortborn bulls have been numbered serially since the establishment of the herdbook. Cows have been numbered since vol. 69 was issued, but previous to that time the volume and page numbers were used to designate them. The name of an animal is not complete unless the number is included, or in the case of cows registered in vols. 1 to 69, the volume and page numbers. Thus Ruby Oakland 6th (vol. 69, p. 920) definitely designates the Ruby Oakland 6th as the one recorded on p. 920 of vol. 69.

3. In vol. 70 and subsequent volumes only the sire and dam of each individual are given. Previous to vol. 70, however, the breeding was recorded in a different fashion, thus:

> 271003 Faultless Perfection Second,
> Red, calved May 17, 1906, bred by William Kiddoo, La Plata, Mo., got by Prince Gloster 199051, out of Miss Dewey 2d (vol. 53, p. 804) by Royal King, Jr. 137701—tracing to imp. Pansy by Blaize (76).

This means that the sire of Faultless Perfection 2d 271003 was Prince Gloster 199051; his dam, Miss Dewey 2d (vol. 53, p. 804), and his dam's sire, Royal King, Jr. 137701. The note, "tracing to imp. Pansy by Blaize (76)," simply means that if the pedigree were followed out continuously along the dam's side, the cow Pansy by Blaize (76) would appear as the cow which was imported from the British Isles. In longer pedigrees like this one, the dash should be read "out of" and refers to the preceding dam.

> 54623 Baron Cloud,
> Red, little white, calved May 16, 1880, bred by S. S. Tipton, Mineral Point, Kans., got by Grand Baron 35687, out of Bertha Cloud (vol. 13) by Red Cloud 10721—Lady Bolt by Ben Bolt 4576—Lady Sheffield by imp. Sheffielder 961½—etc.

Thus Lady Bolt was the dam of Bertha Cloud, Lady Sheffield of Lady Bolt, and so on.

4. English numbers of bulls are distinguished by including them in parentheses; English numbers of cows by the letter E following the volume and page numbers. The volumes of the English herdbook are not necessary for tracing out pedigrees, because the pedigree of all English

Shorthorns to which American bred animals trace are given in the American herdbook. As for instance the pedigree of Pride of the Isles 45274 (35072), an English bull, is given under the American number 45274. A list of the American numbers of some of the older English bulls is given in vol. 28 and in some of the preceding volumes of the herdbook. Pedigrees of cows recorded in the English herdbooks, as indicated by the E following the name in the American herdbooks, can often be traced as for the ancestors of Souvenir in the following:

77932 Spartan Hero (50502)
Red, calved March 23, 1883, bred by Amos Cruickshank, Sittyton, near Aberdeen, Scotland, imported in 1883 by James I. Davidson, Balsam, Ont., owned by D. Cookson and Sons, Downey, Iowa, got by Barmpton 45246, out of Souvenir (vol. 27, p. 362 E) by Royal Duke of Gloster 20901, etc., as in 49937.

In the pedigree of the bull whose number is the last given above, 49937, will be found other ancestors of Souvenir. From that point the ancestry may be traced still further back in the same way.

5. Trace out the pedigree of an animal assigned to you from the following list to the 6th generation. Make out a rough copy first and then recopy it, following the pedigree shown on p. 598 of the text-book as a model.

6. Compute the coefficients of inbreeding and relationship for each ancestral generation as explained on pp. 598–601 of the text-book. See also Pearl and Miner, Tables for Calculating Coefficients of Inbreeding, Maine A. E. S. *Bull.* 218 (1913).

TABLE VIII.—SELECTED LIST OF SHORTHORN BULLS IN AMERICAN HERDBOOKS

Marion Marshall 402366
Crystal Stamp 402629
Gloster Lavender 402630
Golden Memory 402631
Indian Chief 405617
Avondale's Pride 405656
Count Lavender 5th 405657
Archer's Glory 402661
Duke 405678
Keewaydin Goods 405712
Marshal A 405716
Royal Goods 405727
Premier Goods 405742
Fayette Sultan 405782
Red Goods 405810
Red Sinnissippi Duke 405818
Justice Marshal 405841
Canova Hero 405848
Bonnie's Avondale 408197
Cumberland Goods 408256
Baron Sultan 408293
Field Marshal 408330
Gloster King 408345
Woodland Sultan, Jr. 408354
Orange Goods 408365
Carterhall Sultan 408479
Victor Goode 408512
Golden Archer 408528
Starlight 408572
White Seal 408596
Baron Master 408600
Butterfly Sultan 408643
Captain Archer 408682
Royal Diamond 408691
Double Goods 408788
Gloster Knight 408920
Spicy Gloster 2d 457470
Red Coronet 6th 457493
Gloster Sultan 457600
Rex Duke 457725
King George 457825
Leroy 457945
Belmont 457996
Roan Gloster 458215
Choice Duke 458339
Jennie's Boy 458446
White Marvel 458660
Ringmaster 458798
Favorite 458859
Royal Secret 459094
Lord Craven 459087
Imperial 459322
Red Knight 459389
Royal Victor 461145
Haptonian 410432

TABLE VIII—*Continued*

Royal Victor 405866
Cumberland Hero 405883
Shadylawn Sultan 405902
Double Sultan 405911
Red Fashion 405966
Wayne 405995
Roan Orphan 406008
Scotch Prince 406023
Silver King 406054
Beauford Sultan 406061
King Goods 406184
Lord Cumberland 406196
Secret Goods 406201
Gloster Monarch 406229
Beckman's Choice 406239
Harold Lad 406258
Orange Lad 406271
Proud Sultan 406330
Count Sultan 406480
Chancellor 406505
College Chief 406614
Roan Goods 406625
Red Ringmaster 406750
Master Sultan 406758
Gauntlet Goods 406780
Sultan Archer 406880
Red Goods 406905
King Sultan 406908
Golden Bud 406980
Sultan's Choice 406999
Red King 407132
March Archer 407261
Archer's Pride 407216
Missie's Sultan 407217
Bapton Hero 407605
Roan Chief 407614
Gloster Goods 407819
Ceremonious Victor 4th 407832
Oakland Goods 406640
Columbia Master 406668
Violet's Master 406682
Roan Sultan 2d 406707
Golden Goods 407873
Columbia Duke 408093
Matchless Sultan 408106
Sinnissippi Fly 408151
Roan Victor 409550
Lakeside Sultan 409554
Roan Duke 409559
Red Victor 409598
Sultan's Corn 409601
Choice Sultan 409692
Orange Goods 2d 409701
Roan Marshal 409704
Roan Sultan 409742
Prince Cumberland 409781
Matchless Sultan 409795
Scotch Lad 2d 409826
Golden Royal 409851
Ceremonious Paul 409864
Lincoln Marshal 409963
Richmond Lad 409982
Marshal Fame 406723
Thankful Sultan 2d 409989
Roan Goods 410001
Vain Valentine 410042
Lad's Diamond 410043
Prince Cumberland 410147
Alice's Goods 410169
Southfork Lad 410170
Young Sultan 410259
Scotch Victor 410262
Dale's Farewell 410275
Sultan's Coronet 410290
Avon Duke 410304
Chief Archer 410328
Baron Violet 410339
Golden Eagle 410355
Parkdale Baron 410363
Choice Goods 410378
Baron Lavender 410401
The Defender 410418
Archer Jr. 3d 410431
Count Royal 410321
Avondale's Pride 405656
Red Sinnissippi Duke 405818
Gloster Monarch 406229
Victor Goods 408512
The Red Knight 457001
White Knight 457042
Red Archer 457102
Beautiful Avondale 410467
Royal Cumberland 410470
Oakview Lavender 410496
Choice Archer 410514
White Sultan 410522
Gallatin Avondale 410546
Red Sultan 410592
Prince George 410696
Cup's Avondale 410737
Spruce Goods 410874
Cumberland Knight 410875
Bapton Archer 411028
Baron Rosen 2d 411029
Baron Secret 411030
Red Lad 411076
Scottish Goods 411190
Sultan Lad 411217
Prince Victor 411391
Stamford Sultan 411455
Duke of Gloster 411465
Earl Sultan 411641
Browndale Memory 411712
Knight's Goods 411719
Roan Goods 411745
Velvet Sultan 411880
Springcreek Archer 411935
Square Goods 461931
Merry Sultan 463082
Gloster Alexander 463632
Peer 464517
Iron Sides 465292
El Toro 465777
Royal Silver 2d 466631
Roan Lad 468076
George Washington 470098
Chris 470733
Joseph A 462627
Roan Monarch 463340
Red Chief 463836
Sterling Goods 465124
Gloster 465342
Nellie 3d's Dale 466154
Comet 467544
Scotch Harold 469056
Adrian 470422

APPENDICES

APPENDIX I

TABLE IX.—SHOWING THE PROBABILITY OF OCCURRENCE OF STATISTICAL DEVIATIONS OF DIFFERENT MAGNITUDES RELATIVE TO THE PROBABLE ERROR. (*From Pearl*)

Deviation / P. E.	Probable occurrence of a deviation as great as or greater than the designated one in 100 trials	Odds against the occurrence of a deviation as great as or greater than the designated one
1.0	50.00	1.00 to 1
1.1	45.81	1.18 to 1
1.2	41.83	1.39 to 1
1.3	38.06	1.63 to 1
1.4	34.50	1.90 to 1
1.5	31.17	2.21 to 1
1.6	28.05	2.57 to 1
1.7	25.15	2.98 to 1
1.8	22.47	3.45 to 1
1.9	20.00	4.00 to 1
2.0	17.73	4.64 to 1
2.1	15.67	5.38 to 1
2.2	13.78	6.26 to 1
2.3	12.08	7.28 to 1
2.4	10.55	8.48 to 1
2.5	9.18	9.89 to 1
2.6	7.95	11.58 to 1
2.7	6.86	13.58 to 1
2.8	5.90	15.95 to 1
2.9	5.05	18.80 to 1
3.0	4.30	22.26 to 1
3.1	3.65	26.40 to 1
3 2	3.09	31.36 to 1
3.3	2.60	37.46 to 1
3.4	2.18	44.87 to 1
3.5	1.82	53.95 to 1
3.6	1.52	64.79 to 1
3.7	1.26	78.37 to 1
3.8	1.04	95.15 to 1
3.9	.853	116.23 to 1
4.0	.698	142.26 to 1
4.1	.569	174.75 to 1
4.2	.461	215.92 to 1
4.3	.373	267.10 to 1
4.4	.300	332.33 to 1
4.5	.240	415.67 to 1
4.6	.192	519.83 to 1
4.7	.152	656.89 to 1
4.8	.121	825.45 to 1
4.9	.095	1,051.63 to 1
5.0	.074	1,350.35 to 1
6.0	.0052	19,230 to 1
7.0	.00023	474,782 to 1
8.0	.000000068	1,470,588,234 to 1

Elderton's Table for Testing Goodness of Fit will be found in Pearson's *Tables for Biometricians and Statisticians*, pp. 26–28. As permission for the republication of this table cannot be obtained it is suggested that the instructor have it manifolded for the use of his students. If mimeographed the sheets can be kept in the laboratory note book. If photographed the (thin) prints can be pasted in the back of this manual. It would be ridiculous to require elementary students to purchase Pearson's book of tables; yet it is highly desirable that students of genetics become familiar with the use of Harris' formula in testing Mendelian ratios.

APPENDIX II

Rearing Drosophila melanogaster (ampelophila) for Class Use and Research

Obtaining Strains of Drosophila.—The vinegar fly is a cosmopolitan species and wild flies can easily be secured by exposing fermenting fruit. If reared in large numbers and kept under observation for considerable time there is likelihood of discovering mutant individuals which would furnish excellent material for research. For testing such mutants, however, and especially for elementary class instruction it is highly desirable that strains of known genetic constitution be used. Such strains can be secured by applying to any one of the following institutions. It is suggested that instructors apply at the nearest place. Department of Zoology, Columbia University, New York City; Department of Animal Industry, University of Illinois, Urbana; Division of Genetics, University of California, Berkeley.

Culture Vials.—For the student's experimental cultures 1-ounce, (30-c.c.) homeopathic wide-mouthed (1.8-cm.) vials are used. In each clean vial is placed a folded strip of towel paper about three-fourths of an inch wide and 5 to 6 inches in

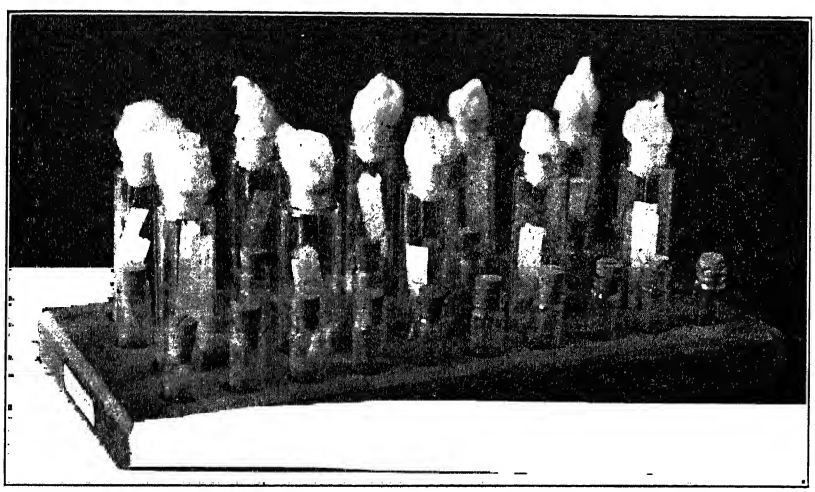


FIG. 13.—Tray for holding culture and isolation vials for Drosophila breeding experiments

length. It is then fitted firmly but not packed with a raw cotton stopper and dry sterilized for 1 hour at 140° to 150°C. Each student is furnished with a wooden tray (see Fig. 13) for the necessary vials. This tray should bear a label with the student's name, laboratory section and desk numbers. For general stock cultures pint milk bottles may be used.

Preparation of Food.—In all operations due care should be exercised to prevent bacterial and fungal infection of the culture media. Fermenting banana has been found to give the most universal satisfaction as a culture medium. For classes of 60 to 150 students the banana may be prepared in 2-quart wide mouth glass fruit jars. With two such jars sufficient material can be prepared for the use of a class of from 60 to 100 students.

The glass jars should be sterilized in water at about 90°C. If the laboratory is fitted with running hot water this sterilization may be done by letting the hot water run into the jar for 30 to 60 minutes. One jar will hold from ten to twelve bananas. They should be peeled and sliced into sections about one-half to three-fourths of an

inch in thickness, which when cut through the center makes a convenient size to place in the 1-ounce vials. Run water, preferably warm, into the jar until it just covers the bananas. Place the jar with lid placed on loosely or with a folded towel across the top in an Anrold sterilizer or an autoclave and sterilize at about 100°C. for 15 to 25 minutes then remove and allow it to cool. If a steam sterilizer is not at hand, the jar of bananas may be placed in a two or two and one-half gallon galvanized pail and placed over a slow bunsen flame so that the water in the pail is kept at the steaming point for one hour. This has been found quite satisfactory, but care must be taken not to overheat the bananas as they will become too soft for convenient use. When the bananas are cool add the yeast solution made by placing about one-fifth of a cake of compressed yeast or about one-fourth of a cake of dried yeast in one-half tumbler of luke warm water. When prepared in this way the compressed yeast can be used 1 hour after placing in the water; the dried yeast requires a longer time to reach best conditions for use. After the yeast solution has been added thoroughly clean and dry the outside of the jar, put the lid an loosely, invert an eight pound paper bag over it tucking the end under the bottom of the jar and place the jar thus protected in an incubator or other place where the temperature is between 25° and 30°C. After remaining at this temperature for about 12 hours it is ready for use and should be removed to a cooler place in order to check the too rapid growth of the yeast. If left too long in the warm temperature the pieces of banana become so soft as to lessen its efficiency as food for the fly larvæ and this also increases the difficulty in placing the food in the culture vials. At optimum conditions the sections of banana are almost as firm as in the raw condition. The bananas used should not be overripe.

Filling the Vials.—To remove food from the stock jar a large strong dissecting needle with curved point is very useful. The needle before being used, however, should be passed through a bunsen flame. With this instrument sections of banana are removed and placed on a piece of sterilized towel paper. Each section is cut in half and with a pair of broad-pointed forceps, which have been flamed, placed between the two ends of the paper strip in the vial. Ordinarily both halves of a section are placed in one vial. The cotton plug which is held between the first and second fingers of the hand holding the vial, is replaced immediately after the food is inserted. The pieces of banana may be firmly settled to the bottom of the vial by striking the bottom of the vial against the palm of the hand.

The preparation and handling of the stock supply of food should be done by the instructor or some other competent person inasmuch as it would be difficult to avoid contamination if each student were to secure his own material from a common stock supply. During the time Drosophila experiments are in progress the person in charge of the stock supply of food should each day put food in about 50 vials (the number depending on the number of experiments being carried) and place them in the laboratory where students may secure them. For feeding student cultures that have been started, the food may be removed from the supply vial with dissecting forceps and placed in the culture vial. This food supplies moisture while in the fermenting condition, but as it becomes old and the yeast is nearly exhausted there will not be enough moisture to sustain life. This dry condition is best corrected by adding a new supply of fermenting banana to the vial.

Certain kinds of bacteria sometimes infect the cultures and produce a shiny, slimy growth over the surface of the banana. The flies will not breed satisfactorily in such cultures and if transferred to a fresh supply of banana they will carry the bacteria with them. Contaminated cultures should be discarded and no flies or pupa should be taken from them to establish other cultures.

Handling the Flies.—The flies are positively phototropic, younger flies being more responsive than older ones. When it is necessary to open a vial it should be held

horizontally with the mouth away from the window, the plug may then be removed as the flies move toward the other end. If it is desired to remove the flies for examination place another clean dry vial or bottle having the same sized opening, mouth to mouth with the vial or bottle containing the flies and then reverse the

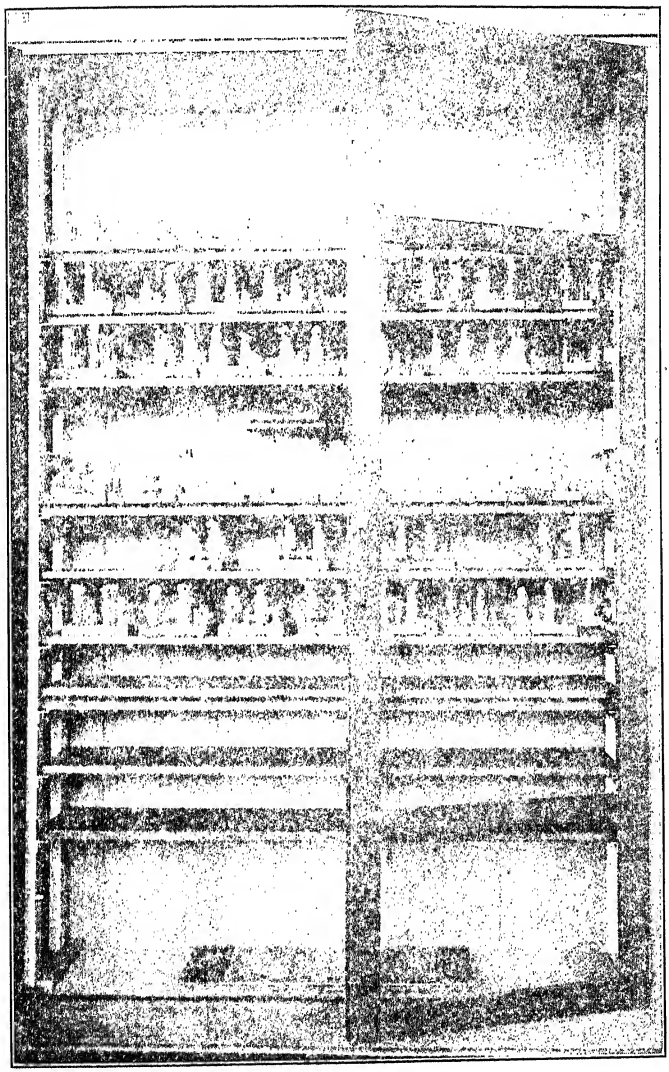


FIG. 14.—Incubator cabinet for student cultures of Drosophila. Note glass doors, metal supports, wooden shelves, electrical heating unit (bottom), thermostat and thermometer (center), switch (right).

two so that the bottom end of the empty vial is toward the source of light and the flies will move into the clean vial. If the flies seem reluctant to leave the culture vial, tapping or jarring the vial will aid in getting them into the empty vial. For

examination, the flies are usually first etherized. For this purpose a clean dry culture vial is fitted up with a cork to which a small felt pad is attached by a wire. First transfer flies from the culture to this clean, dry, vial, then dip the ether pad into ether and cork up the vial with the flies in it. Subject the flies to ether for about 30 seconds after they cease moving about, then empty them out onto a clean sheet of white paper for examination. They will remain quiet 4 or 5 minutes. When properly etherized the wings remain in the normal position and the legs are folded; if overetherized, the wings stand out above and at right angles to the body and the legs are extended. A small soft camel's hair brush should be used for handling the etherized flies.

Isolating Virgin Females.—To obtain virgin females, the culture bottle should be thoroughly emptied of all flies. Six to eight hours later the females which have emerged may then be isolated and used in the matings. Often it is convenient to empty the bottles late in the evening and to take the females out early the next morning. A more accurate method is to place a single pupa in each of several small (5-c.c.) cork-stoppered vials containing a strip of moistened filter paper. Pupæ which are about to emerge should be selected. The flies in the breeding vial may be transferred to a dry vial temporarily while removing the pupæ.

Controlled Temperature.—Although Drosophila can be reared at ordinary room temperature, they develop more rapidly when kept at the optimum temperature (about 25°C). It is therefore advisable to provide incubator cabinets for student cultures and some sort of incubator room or closet for investigational work. A convenient and efficient electrically heated incubator cabinet is shown in Fig. 14.

APPENDIX III

Laboratory materials needed for a course of six Drosophila experiments for 100 students:

Ten dozen small camel's hair brushes.
Five gross wide-mouthed 30-c.c. vials.
Ten gross 5-c.c. vials.
One hundred redwood trays.
Five rolls cotton for plugs.
One hot-air sterilizer.
One Arnold steam sterilizer.
Four 2-quart glass jars.
One dozen pint milk bottles.
One 2½ gallon galvanized iron pail.
Twelve glass-stoppered ether bottles (10-c.c.).
Thirty-five dissecting microscopes or small binoculars.
Bananas: eight dozen during the four months for class use; two dozen per month for keeping strains the remainder of the year.
Ten-thousand fly cuts similar to those shown in Fig. 1 (or some with different wings).
Four pounds ether.
Two rolls or packages paper towelling.
One-hundred Dennison's labels No. 2004.

APPENDIX IV

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